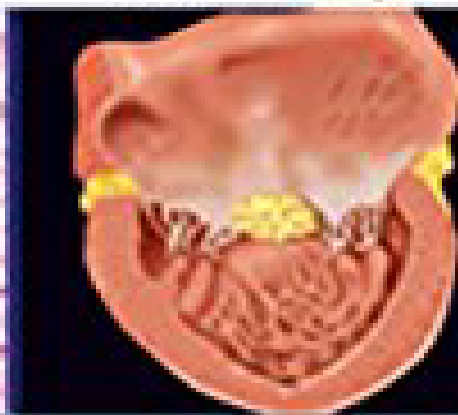
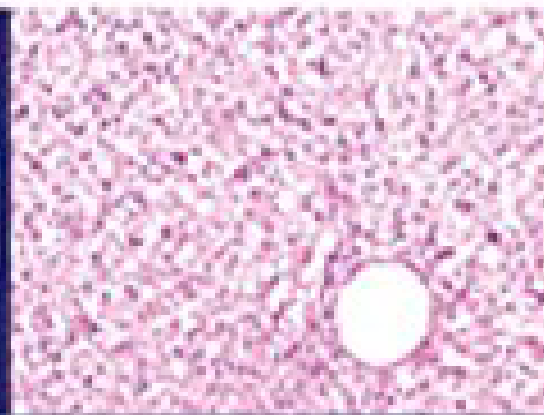




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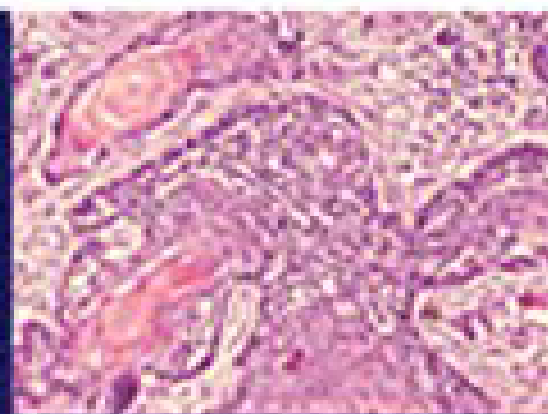
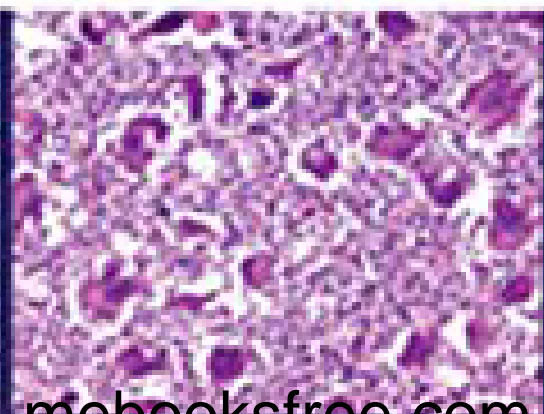


Exam Preparatory Manual for Undergraduates

Pathology

HIGHLIGHTS

- Easy to understand and memorize entire subject including hematology and clinical pathology in short time
- Assured success with high scoring in theory, viva-voce and competitive examination
- Helps both undergraduates and postgraduates
- Recent updates in pathology is covered



Exam Preparatory Manual for Undergraduates

PATHOLOGY

Exam Preparatory Manual for Undergraduates

PATHOLOGY

Second Edition

Ramadas Nayak MBBS MD

Professor and Head
Department of Pathology
Yenepoya Medical College
Yenepoya University
Mangaluru, Karnataka, India
Formerly, Head
Department of Pathology
Kasturba Medical College, Mangaluru
Manipal University
Karnataka, India

Foreword

K Ramnarayan



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Jaypee Brothers Medical Publishers (P) Ltd

Headquarters

Jaypee Brothers Medical Publishers (P) Ltd
4838/24, Ansari Road, Daryaganj
New Delhi 110 002, India
Phone: +91-11-43574357
Fax: +91-11-43574314
Email: jaypee@jaypeebrothers.com

Overseas Offices

J.P. Medical Ltd
83 Victoria Street, London
SW1H 0HW (UK)
Phone: +44 20 3170 8910
Fax: +44 (0)20 3008 6180
Email: info@jpmedpub.com

Jaypee Medical Inc
325, Chestnut Street
Suite 412, Philadelphia
PA 19106, USA
Phone: +1 267-519-9789
Email: support@jpmedus.com

Jaypee Brothers Medical Publishers (P) Ltd
Bhotahity, Kathmandu, Nepal
Phone: +977-9741283608
Email: kathmandu@jaypeebrothers.com

Website: www.jaypeebrothers.com
Website: www.jaypeedigital.com

Jaypee-Highlights Medical Publishers Inc
City of Knowledge, Bld. 235, 2nd Floor, Clayton
Panama City, Panama
Phone: +1 507-301-0496
Fax: +1 507-301-0499
Email: cservice@jphmedical.com

Jaypee Brothers Medical Publishers (P) Ltd
17/1-B Babar Road, Block-B, Shaymali
Mohammadpur, Dhaka-1207
Bangladesh
Mobile: +08801912003485
Email: jaypeedhaka@gmail.com

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Dedicated to

*Students who inspired me,
Patients who provided the knowledge,
My parents and family members,
who encouraged and supported me.*

Foreword



INSPIRED BY LIFE

“Any intelligent fool can make things bigger and more complex,” said Albert Einstein. To make things understandable and appealing is the persisting and daunting task of a passionate teacher. It is in this context that Dr Ramadas Nayak’s book assumes a considerable significance. In this book, he has provided conceptual clarity that it is both astounding and amazing.

The veritable qualities of a review book include simplicity, structure, sequence, and standardization. To this, must be added another ‘s’ i.e. sympathy—sympathy for the reader who is grappling with the essentials. Dr Nayak’s endeavor to have all these qualities in the book is a testimony of his expertise and experience as an effective and exemplary teacher.

I am delighted to write this Foreword to *Exam Preparatory Manual for Undergraduates—Pathology*, which, I am certain, will be an invaluable resource for students and teachers in pathology.



K Ramnarayan

MBBS MD (Path) PG Dip. Higher Education

Vice-Chancellor

Manipal University

Manipal, Karnataka, India

Preface to the Second Edition

Pathology is a rapidly-expanding and ever-changing field and lays the foundation for understanding diseases. This book is an endeavor to present the vast knowledge of pathology in a lucid manner for undergraduate medical students and those undergoing training in paramedical courses. The main aim of this book is to provide a sound knowledge of pathology and hence give insight into etiology, pathogenesis, pathology and the disease course. Every attempt has been made to present information in a simplified text augmented with the use of colored illustrations, tables, text boxes and flowcharts. I have the pleasure of presenting the second edition of book which has become popular within a few months of publishing the first edition titled *Exam Preparatory Manual for Undergraduates—Pathology*. There was sincere request from all students, staffs, my friends and colleagues to include hematology section and nutritional disorders. Hence, hematology and clinical pathology is added as a new Section 2 and nutritional disorders as Chapter 9. There was a tremendous increase in the understanding of molecular pathology and same is highlighted in all the relevant chapters. Thus, this edition is completely revised, updated, better illustrated and a complete manual for scoring high marks in all pathology examinations. In a few chapters, figures and illustrations have been replaced by better quality photomicrographs or illustrations.

Organization

This book consists of 28 chapters and is organized into three sections namely general pathology, hematology and clinical pathology, and systemic pathology.

Section 1—General pathology: It provides an overview of the basic pathologic mechanisms underlying diseases including cellular adaptations, inflammation, wound healing, chronic inflammation, hemodynamic disorders, immunological disorders, neoplasia, genetics and nutritional disorders.

Section 2—Hematology and clinical pathology: It consists of disorder of red cells, disorder of white cells and disorders of hemostasis and clinical pathology essential for the undergraduate students. This was an additional section which was not presented in the first edition of the book. With its introduction, this book becomes a complete exam manual for all students. However, students are requested to go through the second edition of the book titled *Essentials in Hematology and Clinical Pathology* authored by Dr Ramadas Nayak and Dr Sharada Rai for detailed knowledge of hematology and clinical pathology.

Section 3—Systemic pathology: It deals with systemic pathology with chapters devoted to diseases of various organ systems including vascular, cardiac, respiratory, gastrointestinal, liver and biliary tract, pancreas, kidney, male and female genital tract, bones, endocrines, skin and central nervous system.

After many years (more than 36 years) of teaching undergraduates, I found that undergraduate students find it difficult to understand, remember and answer the questions during examinations, in a satisfying way. There are many pathology textbooks, but undergraduates face difficulty to refresh their knowledge during examinations. This book fills the niche, to provide basic information to an undergraduate in a nutshell. The text provides all the basic information the student will ever need to know. Keywords are shown in bold words so that student can rapidly go through the book on the previous day or just before the examination. Most students are fundamentally “visually oriented”. As the saying “one picture is worth a thousand words”, it encouraged me to provide many illustrations.

How to use this book

I recommend that this book to be used by all students for understanding the basic knowledge and refresh their knowledge during examinations. The readers are requested to give more emphasis on word in bold letters which represents the

key words to be remembered. One of the aims of the students after getting undergraduate degree is to fetch a good ranking in the postgraduate entrance examination. Most graduates cannot answer multiple choice questions (MCQs) in entrance examination by just reading the usual textbooks in pathology. In this book, text boxes have been designed to provide information useful in answering these MCQs. Commonly expected pathology questions during undergraduate examination is also provided at relevant places. This book can serve as a source of rapid review of pathology for even postgraduates in pathology.

Numerous illustrations, gross photographs, photomicrographs, tables, text boxes, flow charts and X-rays have been incorporated for easy understanding of the subject. Appendices provide various important bodies and its associated conditions and important cells in various lesions and pathognomonic structures in diseases. In this edition Appendix 3 is included for the reference values of various common and important laboratory tests.

Ramadas Nayak

Preface to the First Edition

Pathology is a rapidly-expanding and ever-changing field and lays the foundation for understanding diseases. This book is an endeavor to present the vast knowledge of pathology in a lucid manner for the second year medical and dental students, and those undergoing training in paramedical courses. My aim is to provide a sound knowledge of pathology and hence give insight into etiology, pathogenesis, pathology and the disease course. Every attempt has been made to present information in a simplified text augmented with the use of colored illustrations.

Organization

This book consists of 23 chapters and is organized into two sections namely general pathology and systemic pathology.

Section 1—General pathology: It provides an overview of the basic pathologic mechanisms underlying diseases including cellular adaptations, inflammation, tissue repair, chronic inflammation, hemodynamic disorders, immunological disorders, neoplasia and genetics.

Section 2—Systemic pathology: It deals with chapters devoted to diseases and disorders of various organ systems including vascular, cardiac, respiratory, gastrointestinal, liver and biliary tract, pancreas, kidney, male and female genital tract, bones, endocrines, skin and central nervous system. For hematology section, readers are requested to refer to *Rapid Review of Hematology* authored by Dr Ramadas Nayak and Dr Sharada Rai and *Essentials in Hematology and Clinical Pathology* by Dr Ramadas Nayak, Dr Sharada Rai and Dr Astha Gupta.

After many years (more than 34 years) of teaching undergraduates, I found that undergraduate students find it difficult to understand, remember and answer the questions during examinations, in a satisfying way. There are many pathology textbooks, but undergraduates face difficulty to refresh their knowledge during examinations. This encouraged me to write a book to fill the niche, to provide basic information to an undergraduate in a nutshell. The text provides all the basic information the student will ever need to know. Keywords are shown in bold words so that student can rapidly go through the book on the previous day or just before the examination. Most students are fundamentally “visually oriented.” As the saying “one picture is worth a thousand words,” it encouraged me to provide many illustrations.

How to use this book

I recommend that this book to be used by all students for understanding the basic knowledge and refresh their knowledge during examinations. The readers are requested to give more emphasis on word in bold letters that represents the key words to be remembered. One of the aims of the students after getting undergraduate degree is to fetch a good ranking in the postgraduate entrance examination. Most graduates cannot answer multiple choice questions (MCQs) in entrance examination by just reading the usual textbooks in pathology. In this book, text boxes have been designed at the sides of main text that provide information useful in answering these MCQs. These boxes also provide commonly expected pathology questions during undergraduate examination. This book can serve as a source of review of general and systemic pathology for even postgraduates in pathology.

About 350 illustrations, 82 gross photographs, 162 photomicrographs, 152 tables, 3 X-rays and a clinical photograph have been incorporated for easy understanding of the subject. Appendices provide various important bodies and its associated conditions, important cells in various lesions and pathognomonic structures in diseases.

Ramadas Nayak

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- Dr Jagadish Rao PP, MBBS, MD, Diplomate NB, PGDCFS, Dip. Cyber Law, PGCTM, MNAMS, District Medicolegal Consultant (Government Wenlock District Hospital), and Associate Professor, Forensic Medicine and Toxicology, Kasturba Medical College (Affiliated to Manipal University), Mangaluru, Karnataka, India.
- Dr Seethalakshmi NV, Professor, Department of Pathology, Amrita Institute of Medical Sciences, Ponekkara PO, Kochi, Kerala, India.
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- Dr Annie Jojo, Professor and Head, Department of Pathology, Amrita Institute of Medical Sciences, Ponekkara PO, Kochi, Kerala, India.
- Dr Raghupathi AR, Former Professor and Head, Department of Pathology, Bangalore Medical College and Research Center, Bengaluru, Karnataka, India.
- Dr Sonali Ullal, Associate Professor, Department of Radiodiagnosis, Kasturba Medical College (Affiliated to Manipal University), Mangaluru, Karnataka, India.
- Dr Veena Shenoy, MD, Chief, Pathology and Laboratory Medical Services, VAMC, Jackson, MS.
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- Dr Mahesh H Karigoudar, Professor, Department of Pathology, BLDE University, Shri BM Patil Medical College, Bijapur, Karnataka, India.
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- Dr Maria Frances Bukelo, MD, Lecturer of Pathology, St. John's Medical College, Bengaluru, Karnataka, India.
- Dr Janaki M, MD, DGO, DFWCD, Professor and Head, Department of Pathology, Santhiram Medical College, Nandyal, Kurnool, India.
- Dr Krishna Pasad HV, Assistant Professor, Department of Pathology, Yenepoya Medical College, A constituent of Yenepoya University, Deralakatte, Mangaluru, Karnataka, India.
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1

SECTION

General Pathology

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3. Wound Healing
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Cellular Responses to Stress and Injury

INTRODUCTION

Definition: Pathology is the **scientific study (logos) of disease (pathos)**. It mainly focuses on the study of the structural, biochemical and functional changes in cells, tissues and organs in disease.

Learning Pathology

Study of pathology can be divided into general pathology and systemic pathology.

- **General pathology:** It deals with the study of mechanism, basic reactions of cells and tissues to abnormal stimuli and to inherited defects.
- **Systemic pathology:** This deals with the changes in specific diseases/responses of specialized organs and tissues.

Scientific Study of Disease

Disease process is studied under following aspects.

Etiology

The etiology of a disease is its **cause**. The causative factors of a disease can be divided into two major categories: Genetic and acquired (e.g. infectious, chemical, hypoxia, nutritional, physical). Most common diseases are multifactorial due to combination of causes.

Pathogenesis

It refers to the sequence by which the causative factor/s produces cellular, biochemical and molecular abnormalities following the exposure of cells or tissues to an injurious

agent. Pathogenesis deals with sequence of events that occur in the cells or tissues from the beginning of any disease process. With the present advances in technology, it is possible to identify the changes occurring at molecular level and this knowledge is helpful for designing new therapeutic approaches.

- **Latent period:** Few causative agents produce signs and symptoms of the disease immediately after exposure. Usually, etiological agents takes **some time to manifest the disease** (e.g. carcinogenesis) and this time period is called as the latent period. It varies depending on the disease.
- **Incubation period:** In disorders caused by infectious (due to bacteria, viruses, etc.) agents, the **period between exposure and the development of disease** is called the incubation period. It usually ranges from days to weeks. Most of the infectious agents have characteristic incubation period.

Molecular Pathology

Most of the diseases can be diagnosed by the morphological changes in tissues. But, with the present advances in diagnostic pathology, the diseases can be analyzed by the molecular and immunological approaches. Molecular pathology has revealed the biochemical basis of many diseases, mainly congenital disorders and cancer. These techniques can detect changes in a single nucleotide of DNA. In situ hybridization can detect the presence of specific genes or their messenger RNA in tissue sections or cell preparations. Minute quantities of nucleic acids can be amplified by the use of the polymerase chain reaction. DNA microarrays can be used to determine patterns of gene expression (mRNA).

MORPHOLOGIC CHANGES

All diseases start with structural changes in cells. **Rudolf Virchow** (known as the **father of modern pathology**) proposed that injury to the cell is the basis of all disease. Morphologic changes refer to the **gross and microscopic structural changes** in cells or tissues affected by disease.

Gross

Lesions: Term used for describing the more or less circumscribed pathological changes in tissues and cells produced by disease.

Many diseases have characteristic gross pathology and a fairly confident diagnosis can be given before light microscopy. For example, serous cystadenoma of ovary usually consists of one cystic cavity containing serous fluid; cirrhosis of liver is characterized by total replacement of liver by regenerating nodules.

Microscopy

Light microscopy: Abnormalities in tissue architecture and morphological changes in cells can be studied by light microscopy.

- **Histopathology:** Sections are routinely cut from tissues and processed by paraffin-embedding. The sections are cut from the tissue by a special instrument called microtome and examined under light microscope. In certain situations (e.g. histochemistry, rapid diagnosis) sections are cut from tissue that has been hardened rapidly by freezing (frozen section). The sections are stained routinely by hematoxylin and eosin.
 - **Pathognomonic abnormalities:** If the **structural changes are characteristic of a single disease or diagnostic of an etiologic process** it is called as **pathognomonic**. Pathognomonic features are those features which are restricted to a single disease, or disease category. The diagnosis should not be made without them. For example, **Aschoff bodies** are pathognomonic of **rheumatic heart disease** and **Reed-Sternberg cells** are pathognomonic of **Hodgkin lymphoma** (refer Appendix II).
- **Cytology:** The cells from cysts, body cavities, or scraped from body surfaces or aspirated by fine needle from solid lesions can also be studied under light microscope. This study of cells is known as cytology and is used widely especially in diagnosis and screening of cancer.
- **Histochemistry (special stains):** Histochemistry (refer Table 1.9) is the study of the chemistry of tissues, where tissue/cells are treated with specific reagent so that the features of individual cells/structure can be visualized, e.g. **Prussian blue reaction for hemosiderin**.
- **Immunohistochemistry and immunofluorescence:** Immunohistochemistry and immunofluorescence utilize antibodies (immunoglobulins with antigen specificity) to visualize substances in tissue sections or cell preparations. Former uses **monoclonal antibodies** linked chemically to enzymes and later **fluorescent dyes**.

Electron microscopy: Electron microscopy (EM) is useful to the study changes at ultrastructural level, and to the demonstration of viruses in tissue samples in certain diseases. The most common diagnostic use of EM is for the interpretation of biopsy specimen from kidney.

Functional Derangements and Clinical Manifestations

- **Functional derangements:** The effects of genetic, biochemical and structural changes in cells and tissues are **functional abnormalities**. For example, excessive secretion of a cell product (e.g. nasal mucus in the common cold); insufficient secretion of a cell product (e.g. insulin lack in diabetes mellitus).
- **Clinical manifestations:** The functional derangements produce, clinical manifestations of disease, namely symptoms and signs. Diseases characterized by **multiple abnormalities** (symptom complex) are called syndromes.
- **Prognosis:** The prognosis forecasts (predicts) the known or likely **course (outcome) of the disease** and, therefore, the fate of the patient.
- **Complications:** It is a **negative pathologic process** or event occurring **during the disease** which is not an essential part of the disease. It usually aggravates the illness. For example, perforation and hemorrhage are complications which may develop in typhoid ulcer of intestine.
- **Sequelae:** It is a pathologic condition **following as a consequence of a disease**. For example, intestinal obstruction following healed tuberculosis of intestine, mitral stenosis following healed rheumatic heart disease.
- **Remission and relapse:**
 - **Remission:** It is the process of conversion from active disease to quiescence. Some of the chronic diseases are interspersed by periods of quiescence when the patient is relatively in good health.
 - **Relapse:** It is the process in which the signs and symptoms of disease reappear.

Some diseases may pass through several cycles of remission and relapse. For example, inflammatory bowel disease (Crohn's disease and ulcerative colitis).

TYPES OF CELLULAR RESPONSES TO INJURY

Depending on the nature of stimulus/injury, the cellular responses can be mainly divided into four types (Fig. 1.1).

1. Cellular adaptations
2. Cell injury
 - Reversible cell injury
 - Irreversible cell injury.
3. Intracellular accumulations
4. Pathologic calcification.

Different stages of cellular responses to stress and injurious stimuli are shown in Figure 1.2.

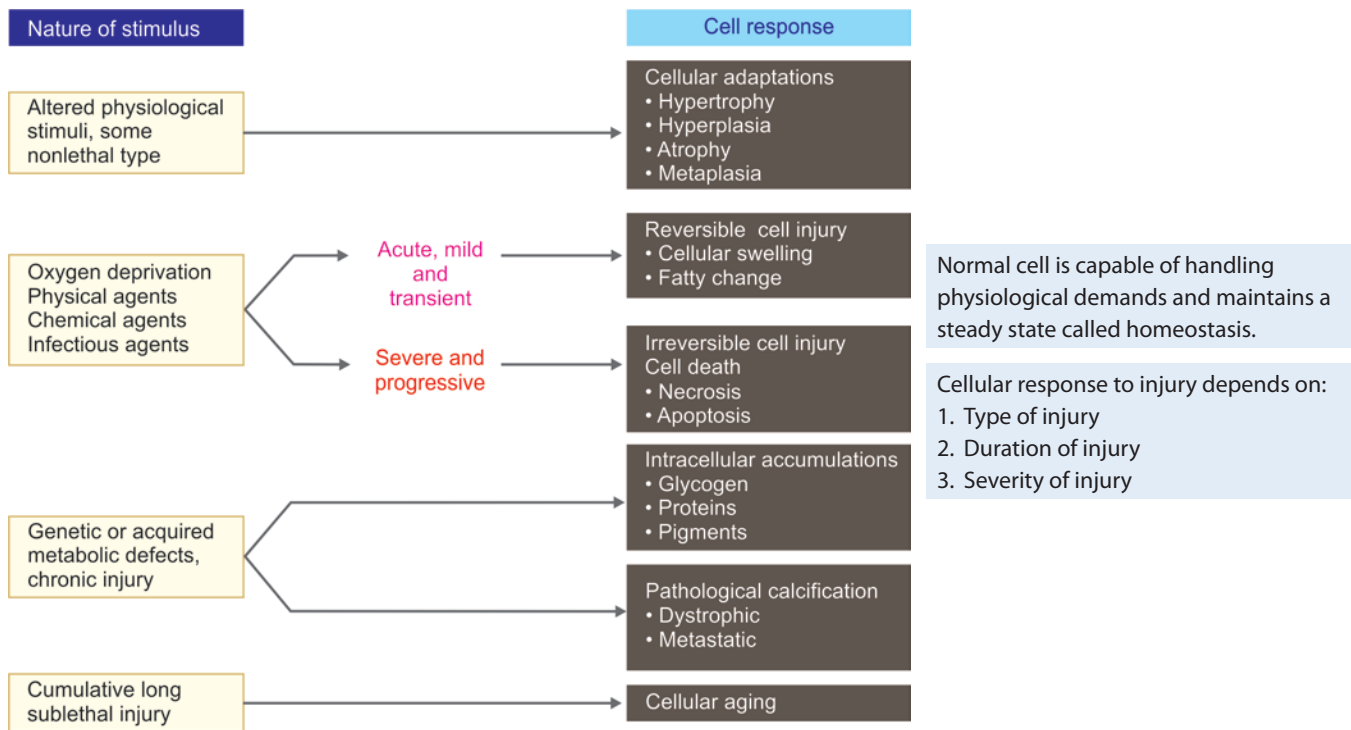


Fig. 1.1: Types of cellular responses to stimuli/injury

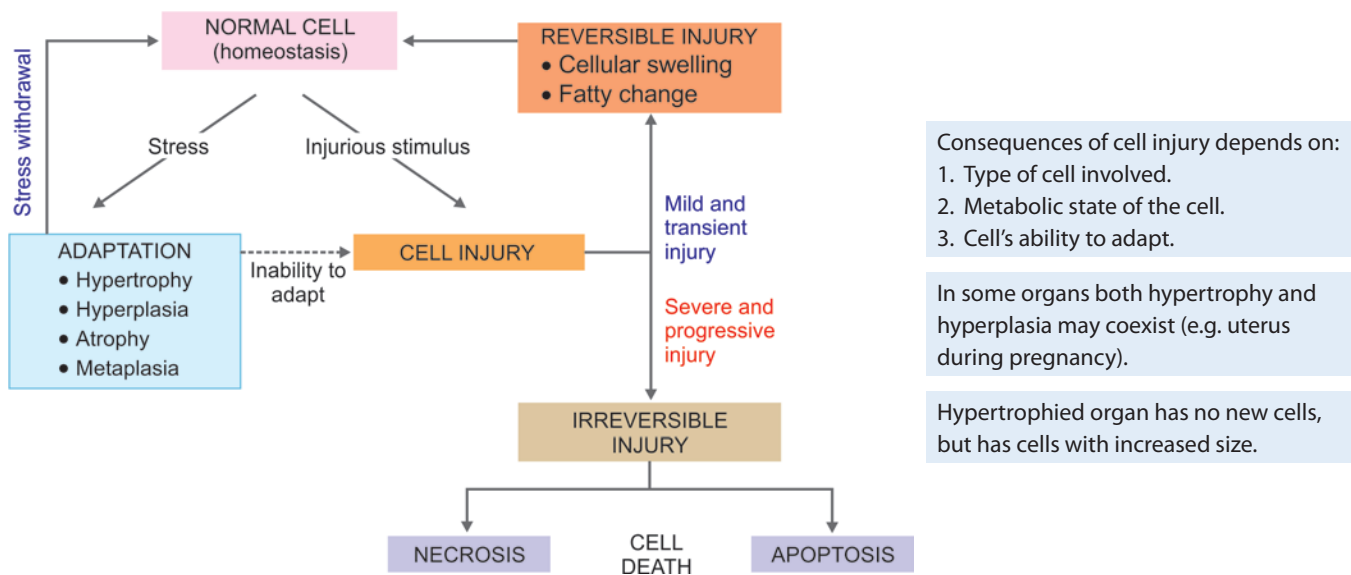


Fig. 1.2: Different stages of cellular responses to stress/injury

CELLULAR ADAPTATIONS

Q. Write short note on cellular adaptations.

When the cell is exposed to pathological stimuli, the cells can achieve a **new, steady altered state that allows them to survive** and continue to function in an abnormal

environment. These are reversible changes in the size, number, phenotype, metabolic activity or functions of cells constitute **cellular adaptations**.

Types of adaptations: Hypertrophy, hyperplasia, atrophy and metaplasia.

Hypertrophy

Q. Write short note on hypertrophy.

Definition: Increase in the size of the tissue or organ due to increase in the size of cells.

Causes

Increased functional demand/workload.

Hypertrophy: Occurs in tissues incapable of cell division.

Physiological

- **Hypertrophy of skeletal muscle:** For example, the bulging muscles of body builders and athletes.
- **Hypertrophy of smooth muscle:** For example, growth of the uterus during pregnancy from estrogenic stimulation.

Pathological

- **Hypertrophy of cardiac muscle:** For example, left ventricular hypertrophy (Fig. 1.3) due to hypertension or damaged valves (aortic stenosis, mitral incompetence).
- **Hypertrophy of smooth muscle:** For example, hypertrophy of urinary bladder muscle in response to urethral obstruction (e.g. prostate hyperplasia Figs 1.5 and 22.5), hypertrophy of muscular layer of stomach due to pyloric stenosis.

Mechanisms of Cellular Hypertrophy

Hypertrophy is due to increased synthesis of cellular proteins. Steps involved in biochemical mechanisms of myocardial (cardiac muscle) hypertrophy are shown in Figure 1.4.

Activation of the Signal Transduction Pathways

Various mechanisms involved are:

Physiologic hypertrophy:

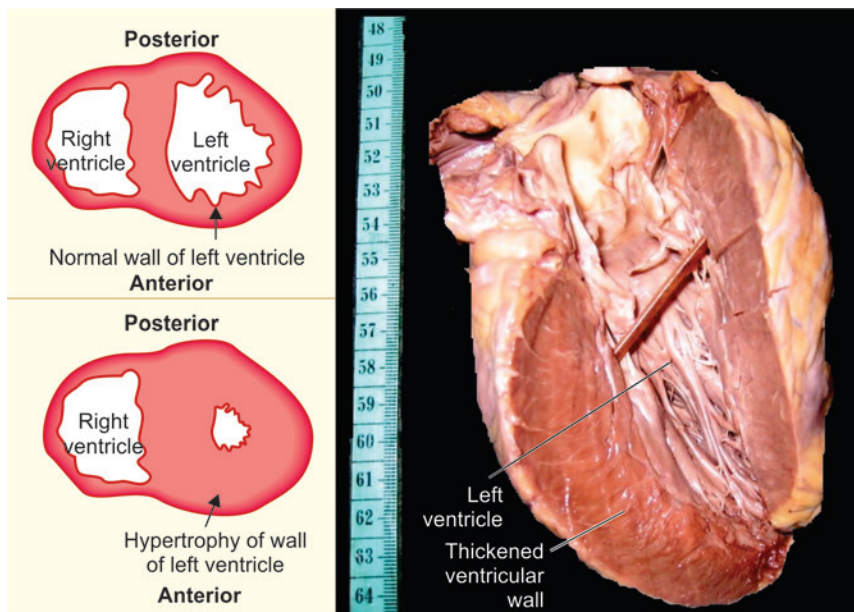
Increased workload on the myocardium produces **mechanical stretch** and is the major trigger for physiological hypertrophy.

Pathologic hypertrophy:

Growth factors and hypertrophy agonists are involved in pathologic hypertrophy.

- **Growth factors:** These include (TGF- β), insulin-like growth factor-1 (IGF-1) and fibroblast growth factor (FGF).
- **Hypertrophy agonists:** These include α -adrenergic agonists, endothelin-1, angiotensin II, nitric oxide (NO), and bradykinin.

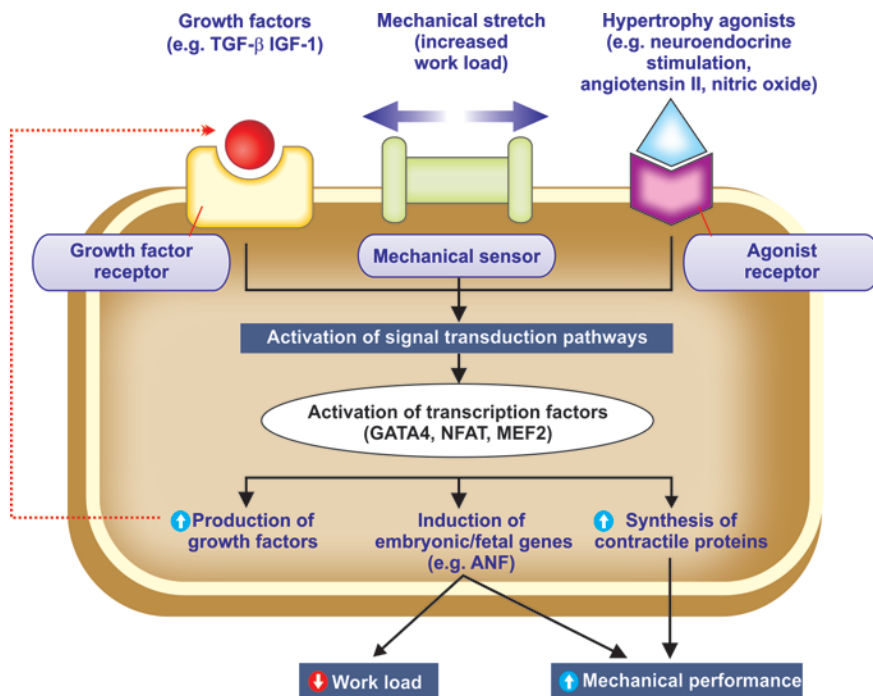
Mechanical sensors also stimulate production of growth factors and agonists. They cause increased synthesis of muscle proteins.



Figs 1.3A to C: (A) Transverse section of normal heart; (B) Transverse section of heart with thickening of wall of the left ventricle due to hypertrophy; (C) Longitudinal section of heart with left ventricular hypertrophy

Hypertrophy:

1. Increased synthesis of contractile proteins
2. Induction of embryonic/fetal genes
3. Increased production of growth factors.



Cardiac hypertrophy:
ANF gene is re-expressed.

Hypertrophy of subcellular organelle
can sometimes occur (e.g. hypertrophy
of the smooth endoplasmic reticulum in
hepatocytes by barbiturates and alcohol).

Fig. 1.4: Mechanisms of myocardial hypertrophy.

Abbreviations: ANF, atrial natriuretic factor; IGF-1, insulin-like growth factor; TGF- β , transforming growth factor- β

Activation of Transcription Factors

Mechanical stretch, growth factors and hypertrophy agonists activate the signal transduction pathways and transcription factors [e.g. GATA4, nuclear factor of activated T-cells (NFAT) and myocyte enhancer factor 2 (MEF2)]. The activated transcription factors results in:

- **Increased synthesis of contractile proteins:** This is necessary to meet the increased functional demand.
- **Induction of embryonic/fetal genes:** Some genes are normally expressed only during early development of embryo and fetus. They are re-expressed in hypertrophied cells. For example, the gene for atrial natriuretic factor (ANF) is expressed in the embryonic heart, but not expressed after birth. In cardiac hypertrophy, ANF gene is re-expressed. ANF is a hormone that causes salt secretion by the kidney, decreases blood volume and pressure. Its re-expression decreases hemodynamic workload and increases the mechanical performance.
- **Increased production of growth factors.**

MORPHOLOGY

- **Gross:** Involved organ is enlarged.
- **Microscopy:** Increase in size of the cells as well as the nuclei.

Hyperplasia

Q. Write short note on hyperplasia.

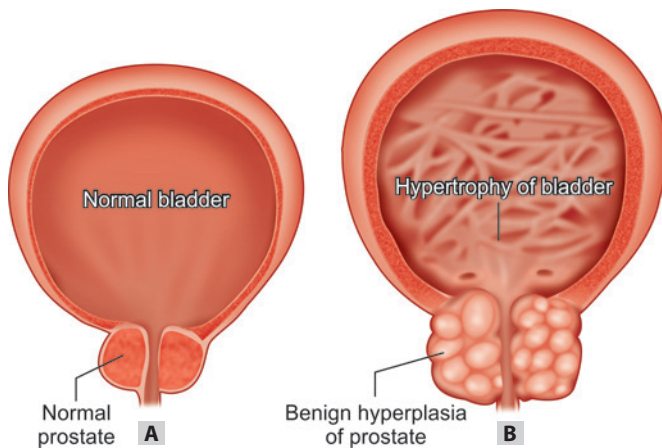
Definition: Increase in the number of cells in an organ or tissue, resulting in increased size/mass of the organ or tissue.

Causes

- **Physiological hyperplasia:** Hormonal stimulation or as compensatory process.
 - **Hyperplasia due to hormones:** For example, hyperplasia of glandular epithelium of the **female breast at puberty, pregnancy and lactation**, hyperplasia of the uterus during pregnancy from estrogenic stimulation
 - **Compensatory hyperplasia:** For example, in liver following partial hepatectomy.

Hyperplasia occurs in cells capable of replication namely labile/stable or stem cells. Not in permanent cells.

- **Pathological hyperplasia:** Due to excess endocrine stimulation or chronic injury/irritation.
 - **Excessive hormonal stimulation:** For example, endometrial hyperplasia (due to estrogen, refer Figs 23.12 and 23.13) and benign prostatic hyperplasia [due to androgens (Figs 1.5 and 22.3 to 22.5)].



Figs 1.5A and B: Cut section of prostate along with urinary bladder: (A) Normal prostate; (B) Enlarged prostate due to nodular hyperplasia. The urinary outflow obstruction results in hypertrophy of bladder muscle

- **Chronic injury/irritation:** Long-standing inflammation or chronic injury may lead to hyperplasia especially in skin or oral mucosa.

Pathological hyperplasia can progress to cancer, e.g. **endometrial hyperplasia** can develop into **endometrial cancer**.

Mechanism

- Hyperplasia is characterized by cell proliferation mostly of mature cell mediated **through stimulation by growth factor or hormones**.
- In some cases, the new cells may be derived from tissue stem cells.

MORPHOLOGY

- **Gross:** Size of the affected organ is **increased**.
- **Microscopy:** Increased number of cells with increased number of mitotic figures.

Atrophy

Q. Write short note on atrophy.

Definition: Atrophy is the **reduced size of an organ or tissue** resulting from a decrease in cell size and number.

Causes

Physiological atrophy: Common during normal fetal development and in adult life.

- During fetal development: For example, atrophy of embryonic structures such as thyroglossal duct.
- During adult life: For example, involution of thymus, atrophy of brain, gonads and heart due to aging (senile atrophy).

Pathological hyperplasia can act as a fertile soil for cancer.

Benign hyperplasia of prostate: Due to action of hormone dihydrotestosterone and not testosterone.

Hyperplasia unlike neoplasia can regress, if the stimulation is eliminated.

Atrophy may be reversible but with irreversible loss of cells and the size of the organ cannot be restored.

Pathological atrophy: Local or generalized.

1. Local

- **Disuse atrophy (decreased workload):** For example, atrophy of limb muscles immobilized in a plaster cast (as treatment for fracture) or after prolonged bed rest.
- **Denervation (loss of innervation) atrophy:** For example, atrophy of muscle due to damage to the nerves (e.g. poliomyelitis).
- **Ischemic (diminished blood supply) atrophy:** For example, brain atrophy produced by ischemia due to atherosclerosis of the carotid artery.
- **Pressure atrophy:** For example, atrophy of renal parenchyma in hydronephrosis due to increased pressure.

In atrophy cell, death is mainly due to apoptosis.

2. Generalized

- **Starvation (inadequate nutrition) atrophy:** For example, protein-calorie malnutrition.

Mechanisms

Atrophic cells have diminished function. There is **decreased protein synthesis and increased protein degradation** in cells.

MORPHOLOGY

- **Gross:** The organ is **small and often shrunken**.
- **Microscopy:** The cells are **smaller in size** due to reduction in cell organelles.

Atrophied cells have increased lipofuscin (wear and tear) pigment.

Differences between atrophy, hypertrophy and hyperplasia are listed in Table 1.1.

Q. List the differences between atrophy, hypertrophy and hyperplasia.

Metaplasia

Q. Write short note on metaplasia with examples.

Definition: Metaplasia is a reversible change in which **one adult cell type is replaced by another adult cell type**.

Causes

- Metaplasia is usually **fully reversible adaptive response to chronic persistent injury**. If the noxious stimulus is

removed (e.g. cessation of smoking), the metaplastic epithelium may return to normal.

- Metaplasia is mainly **seen in association with tissue damage, repair and regeneration**.
- The **replacing cell type** is usually **more suited to a change in environment**.

Types of Metaplasia

Epithelial Metaplasia

Epithelial metaplasia: Most common type of metaplasia.

Squamous metaplasia: Original epithelium is replaced by squamous epithelium.

- Respiratory tract:** For example, chronic irritation due to tobacco smoke, the normal ciliated columnar epithelial cells of the trachea and bronchi undergo squamous metaplasia (Fig. 1.6).

TABLE 1.1: Differences between atrophy, hypertrophy and hyperplasia

	<i>Atrophy</i>	<i>Hypertrophy</i>	<i>Hyperplasia</i>
Definition	Reduced size of an organ or tissue resulting from a decrease in cell size and number.	Increase in the size of the tissue or organ due to increase in the size of cells	Increase in the size/mass of the organ or tissue due to increase in the number of cells
Size of the involved organ	Reduced	Increased/enlarged	Increased
Cells			
• Number	Reduced	No change	Increased
• Size	Reduced	Increased	No change
• Organelles	Reduced	Increased	No change
Rate of cell division	-	-	Increased
Synthesis of DNA, RNA and protein	-	Increased	Increased

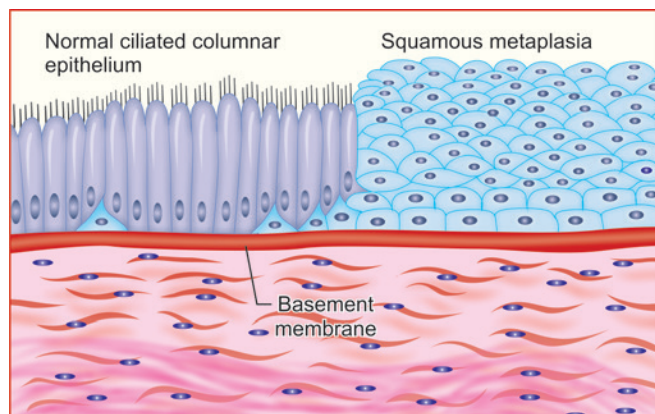


Fig. 1.6: Squamous metaplasia in which columnar epithelium (left) is replaced by squamous epithelium (right)

Persistence of stimulus/stress producing metaplasia may predispose to malignant transformation.

Metaplastic squamous epithelium can withstand the stimulus/stress.

Metaplasia named by the cell which replaces. e.g. squamous metaplasia.

- **Cervix:** Squamous metaplasia in cervix is associated with chronic infection.

Columnar metaplasia: Original epithelium is replaced by columnar epithelium.

- **Squamous to columnar:** In Barrett esophagus, the squamous epithelium of the esophagus replaced by columnar cells (refer Fig. 18.1).
- **Intestinal metaplasia:** The gastric glands are replaced by cells resembling those of the small intestine.

Barrett esophagus: Squamous epithelium of the esophagus is replaced by columnar cells.

Connective Tissue Metaplasia

- **Osseous metaplasia:** Formation of new bone at sites of tissue injury is known as osseous metaplasia. Bone formation in muscle, known as **myositis ossificans**, occasionally occurs after intramuscular hemorrhage. Other examples include cartilage of larynx and bronchi in elderly individual, scar of chronic inflammation of long duration, fibrous stroma of tumor (e.g. leiomyoma).

Connective tissue metaplasia: Myositis ossificans is characterized by bone formation in muscle after trauma.

Mechanism

Develops due to the **reprogramming of precursor cells** (i.e. stem cells or undifferentiated mesenchymal cells) that are present in normal tissues.

Hyperplasia/metaplasia in certain cases may progress to dysplasia and neoplasia.

CELL INJURY

Q. Write short note on causes of cell injury.

Definition: Cell injury is the effect of stresses due to variety of etiological agents on the cell.

Causes of Cell Injury

- A. Hypoxia:** It refers to **inadequate oxygenation of tissue**. It is the **most common cause of cell injury**.

Causes of hypoxia:

- **Decreased blood flow** is called **ischemia**. It may be due to thrombosis, embolism, atherosclerosis or external compression of vessel.
- **Inadequate oxygenation of the blood (hypoxemia)**
 - Due to pulmonary disease.
 - Decreased perfusion of tissues: For example; cardiac failure, hypotension shock.
 - Decreased oxygen-carrying capacity of the blood: For example, anemia.
 - Severe blood loss.

Hypoxia: Most common cause of cell injury.

Ischemia: Most common cause of hypoxia.

Mechanism of injury: Hypoxia causes cell injury by reducing aerobic oxidative respiration and decreasing the synthesis of adenosine triphosphate (ATP).

Outcome: Depending on the severity of the hypoxia, cells may adapt, undergo injury, or die.

Neurons: Most susceptible to hypoxia and irreversible damage occurs 5 minutes after global hypoxia. First cellular change in hypoxia is decreased oxidative phosphorylation in mitochondria.

Watershed areas: Region between terminal branches of arterial blood supply, where blood supply does not overlap. They are susceptible to hypoxic injury. Watershed areas examples:

1. Cerebral vessels
2. Mesenteric arteries.

B. Physical Agents:

- **Mechanical trauma:** For example, blunt/penetrating/crush injuries, gunshot wounds.
- **Thermal injury:** Extremes of temperature (burns and deep cold).
- **Radiation** (ionizing radiation and non-ionizing radiation).
- **Electric shock.**
- **Pressure changes:** Sudden changes in atmospheric pressure.

C. Chemical Agents:

- **Heavy metals and poisons:** For example, arsenic, mercuric salts or cyanide.
- **Simple chemicals:** For example, hypertonic concentrations of glucose or salt.
- **Strong acids and alkalies.**
- **Oxygen at high concentrations is toxic.**
- **Environmental and air pollutants:** For example, insecticides, and herbicides.
- **Industrial and occupational hazards** (carbon monoxide and asbestos).
- **Social/lifestyle choices:** Addiction to drugs and alcohol, cigarette smoking.
- **Therapeutic drugs.**

- D. Infectious Agents:** Viruses, bacteria, fungi, rickettsiae and parasites. The mechanism by which these infectious agents cause injury varies.

E. Immunologic Reactions

- **Autoimmunity:** Immune reactions to endogenous self-antigens are responsible for autoimmune diseases.
- **Hypersensitivity reactions and other immune reactions:** Heightened immune reactions to many external agents (e.g. microbes and environmental agents).

F. Genetic Derangements: Genetic defects may cause cell injury because of:

- Deficiency of functional proteins (e.g. enzyme defects in inborn errors of metabolism).
- Accumulation of damaged DNA or misfolded proteins
- Variations in the genetic makeup.

G. Nutritional Imbalances:

- **Nutritional deficiencies:**
 - Protein-calorie deficiencies
 - Deficiencies of specific vitamins.
- **Nutritional excesses:**
 - Excess of cholesterol predisposes to atherosclerosis.
 - Obesity is associated with increased incidence of several important diseases, such as diabetes and cancer.
 - Hypervitaminosis.

H. Idiopathic: Cause is not known.

General Principles of Cell Injury

1. **Cellular response to injury:** It depends on: (1) type of injury, (2) duration of injury and (3) severity of injury.
2. **Consequences of injury:** It depends on: (1) type of cell involved, (2) adaptability of cell, (3) status of cell and (4) genetic makeup of the cell.

3. **Targets and biochemical mechanism of cell injury:** These include (1) mitochondrial damage/dysfunction, (2) disturbance of calcium homeostasis, (3) damage to cellular membranes and (4) damage to DNA and misfolding of proteins.

Mechanisms of Cell Injury

Q. Write short note on mechanism (biochemical basis) of cell injury.

Injurious stimuli that cause cell injury lead to complex cellular, biochemical and molecular changes. Certain mechanism is common for most forms of cell injury and cell death.

Decreased Production of Adenosine Triphosphate

Adenosine triphosphate (ATP) is required for all processes within the cell. Injury like hypoxia, chemicals (e.g. cyanide) can cause decreased production of ATP.

- **Effects of decreased ATP (Fig. 1.7):**
 - Failure of the cell membrane sodium pump
 - Increased anaerobic glycolysis
 - Failure of the calcium pump
 - Failure of protein synthesis in the ribosomes.

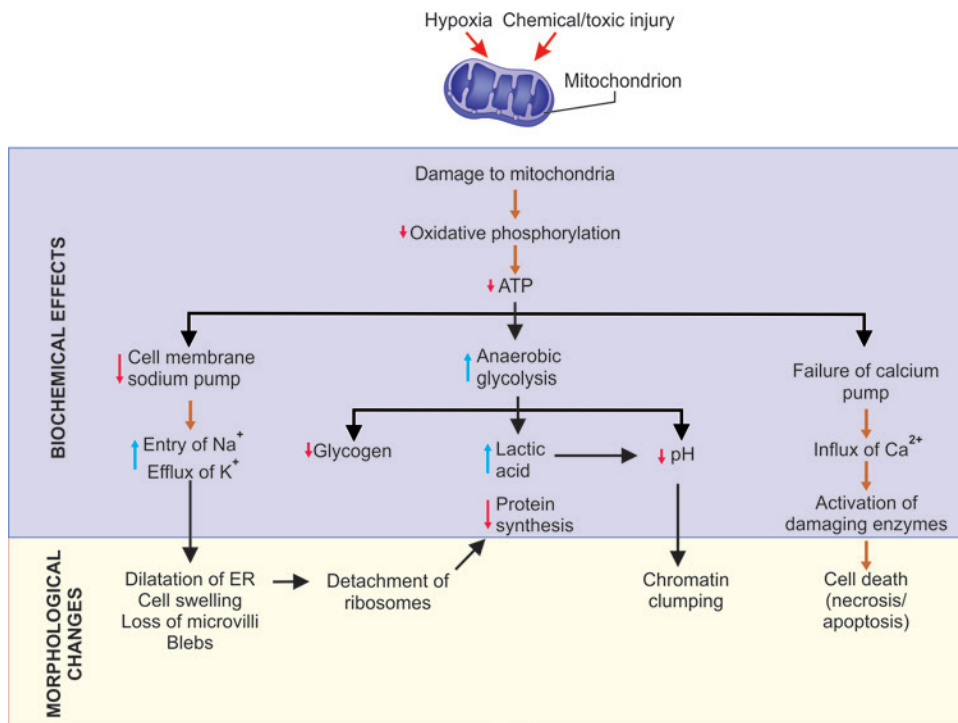


Fig. 1.7: Biochemical and morphological changes due to decreased ATP production

Q. Describe the role of cytosolic calcium in cell injury.

ATP is required for all synthetic and degradative processes within the cell.

Mitochondria:

- Earliest organelle affected in cell injury
- Target for all type of injurious stimuli.

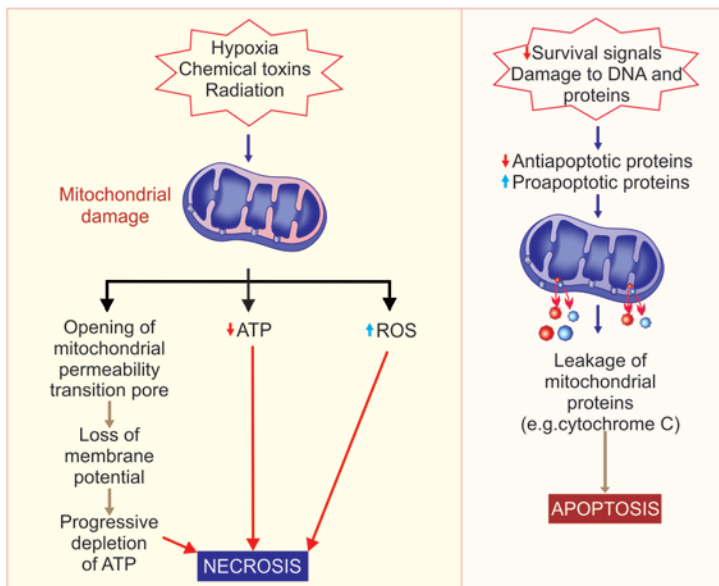


Fig. 1.8: Effects of mitochondrial damage

Oxidation: Loss of electrons.

Reduction: Gain of electrons.

Redox reaction: Reduction-oxidation reaction.

Hydroxyl free radicals are the most powerful free radicals.

Mitochondrial Damage (Fig. 1.8)

- Mitochondria are sensitive to almost all types of injurious stimuli (e.g. hypoxia, toxins).

Consequences of Mitochondrial Damage

- Depletion of ATP:** Its effects are mentioned above.
- Formation of reactive oxygen species (ROS):** Its effects are mentioned in page 13 (refer Fig. 1.10).
- Formation of mitochondrial permeability transition pore:** It occurs in the mitochondrial membrane. This leads to the **loss of mitochondrial membrane potential, pH changes** and **progressive depletion of ATP** and ultimately **necrosis** of the cell.
- Leakage of mitochondrial proteins into cytoplasm:** The mitochondrial membranes contain many proteins **such as cytochrome C and proapoptotic proteins (e.g. BAX and BAK)**. Increased permeability of the mitochondrial membrane may result in leakage of these proteins into the cytosol and induce **apoptosis**.

Influx of Calcium and Loss of Calcium Homeostasis (Fig. 1.9)

Normally, concentration of cytosolic calcium is very low and most of it is sequestered in mitochondria and the endoplasmic reticulum (ER). Ischemia and certain toxins cause an increase in cytosolic calcium (Fig. 1.9). Initially, it is due to the release from intracellular stores and later due to influx across the cell membrane. Increased intracellular calcium stimulates **activation of several damaging enzymes**

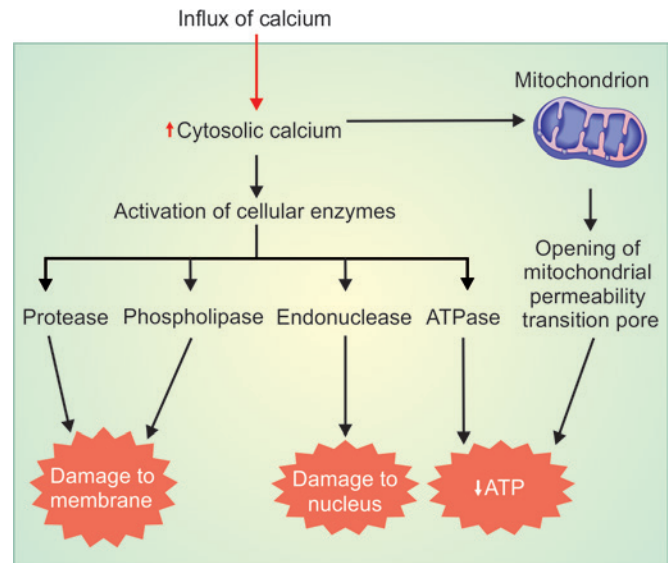


Fig. 1.9: Effects of increased cytosolic calcium in cell injury

(e.g. phospholipases, endonucleases and protease) as well as caspases. The **net result is apoptosis**.

Accumulation of Oxygen-derived Free Radicals (Oxidative Stress)

Q. Write short essay/note on free radical injury and its role in cell injury.

Free radicals are **unstable chemical compounds** with a **single unpaired electron in an outer orbit** (Fig. 1.10).

Properties of Free Radicals

- Normally, **free radicals** produced in the cells are **unstable** and are **rapidly destroyed**.
- When free radicals react with any molecules they convert those molecules into free radicals and thus initiate **autocatalytic reactions**.

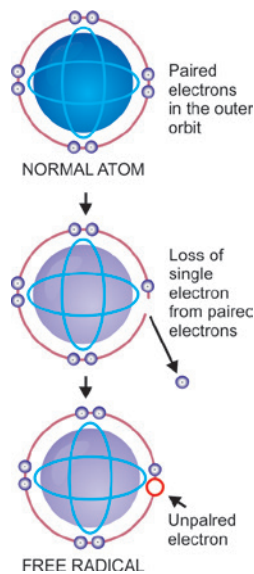


Fig. 1.10: Formation of free radical

- **Excess** of free radicals may be either **due to increased production or ineffective degradation**.

Types of Free Radicals

Q. Write short note on free radical injury.

- **Oxygen-derived free radicals:** Reactive oxygen species (ROS) are oxygen-derived free radicals. ROS includes superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl ions (*OH).
- **Reactive nitrogen species/nitric oxide derived free radicals:** For example, nitric oxide (NO) is generated by endothelial cells (refer Fig. 2.6), macrophages, neurons, and other types of cells. NO can act as a free radical and can also be converted to highly reactive peroxynitrite anion ($ONOO^-$), NO_2 and NO_3^- .
- **Free radicals from drug and chemical:** Enzymatic metabolism of exogenous chemicals or drugs can generate free radicals which are not ROS but have similar effects (e.g. CCl_4 can generate CCl_3^*).

Mechanism of Production of ROS

1. **In all cells** (Fig. 1.11): ROS are produced normally in **small amounts in the mitochondria** during the reduction-oxidation (redox) reactions occurring during mitochondrial respiration and production of energy.

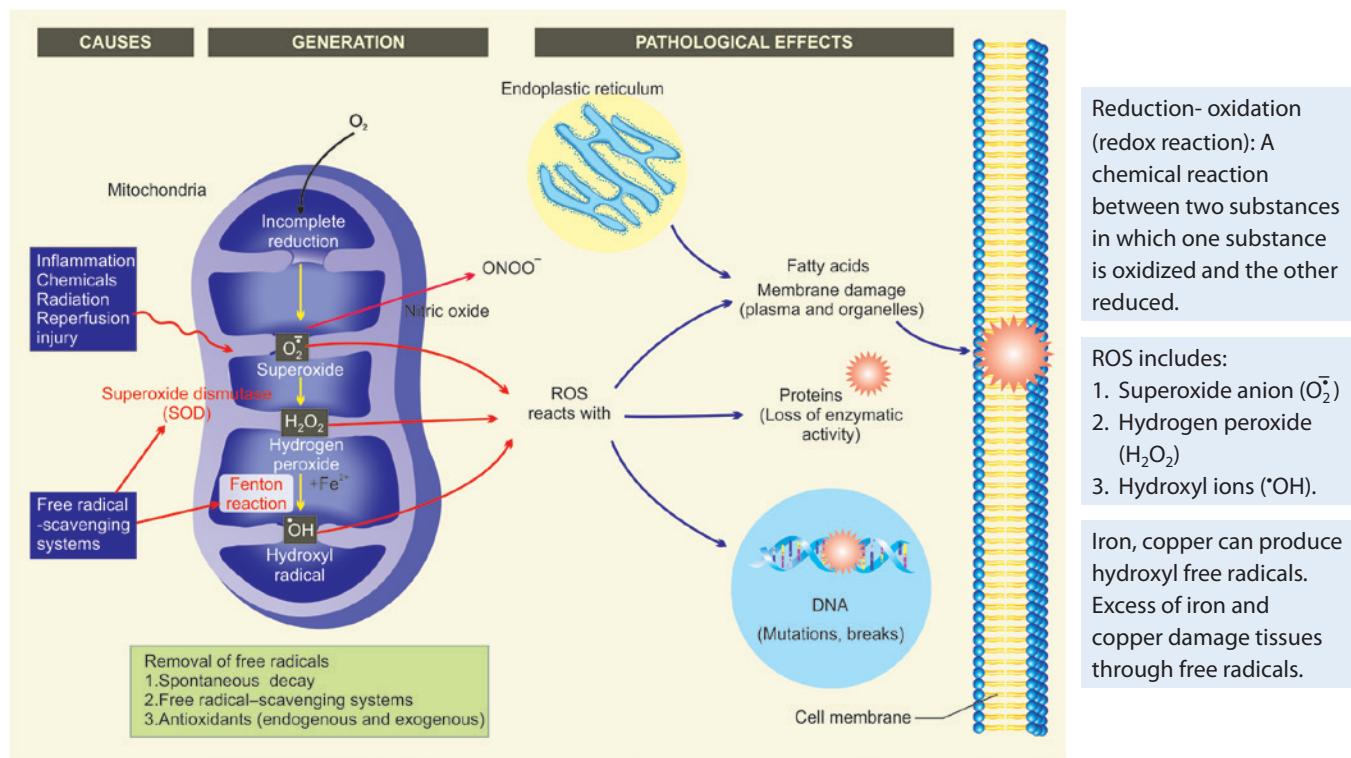


Fig. 1.11: Production of reactive oxygen species in mitochondria of cells and effects in cell injury

- During redox reaction **superoxide (O_2^-)** is produced when oxygen (O_2) is only partially reduced.
 - Superoxide (O_2^-) is converted to **hydrogen peroxide (H_2O_2)** spontaneously and by the action of the enzyme superoxide dismutase (SOD).
 - Hydrogen peroxide (H_2O_2) in the presence of metals (e.g. Fe^{2+}) is converted by Fenton reaction to a highly reactive free radical called **hydroxyl radical ($\cdot OH$)**.
 - Superoxide (O_2^-) is also converted to peroxynitrite ($ONOO^-$) in the presence of nitric oxide (NO).
2. In **phagocytic leukocytes** (Fig. 1.12): ROS produced to destroy the ingested microbes and other substances produced during inflammation.
- During phagocytosis **ROS produced in the phagosomes and phagolysosomes** is formed in the leukocytes (mainly neutrophils and macrophages) by a process similar to mitochondrial respiration. This process is called as **respiratory burst**.
 - Superoxide (O_2^-) is synthesized via NADPH oxidase (nicotinamide adenine dinucleotide phosphate/respiratory burst oxidase) (phagocyte oxidase) present in the phagosome and phagolysosomal membrane of the leukocytes.
 - Superoxide (O_2^-) is converted to **hydrogen peroxide (H_2O_2)**.
 - Hydrogen peroxide (H_2O_2) in the presence of myeloperoxidase enzyme is converted to **highly reactive compound hypochlorite (HOCl)**.

Oxidase reactions produce superoxide free radicals.

Conditions Associated with Increased Generation of Oxygen-derived Free Radicals (Fig. 1.11)

- During inflammation and microbial killing by phagocytes.
- Drugs and chemical injury, including chemical carcinogens.
- Radiation injury (e.g. ultraviolet light, X-rays).
- Reduction-oxidation reactions.
- Ischemia-reperfusion injury induced by restoration of blood flow in ischemic tissue.

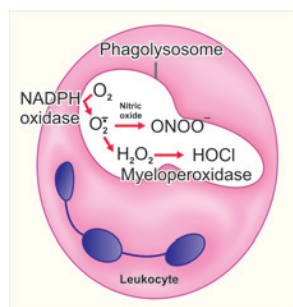


Fig. 1.12: Production of reactive oxygen species in leukocytes

- Transition metals such as iron and copper donate or accept free electrons during intracellular reactions and catalyze free radical formation, as in the Fenton reaction ($H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH + OH^-$).
- Cellular aging.

Free radicals are neutralized by superoxide dismutase, glutathione peroxidase and antioxidants such as vitamin C and E. Vitamin C mainly neutralizes hydroxyl free radicals.

Mechanisms of Removal/Neutralization of Free Radicals (Fig. 1.11)

Q. Write short note on antioxidants.

Serum, tissue fluids and host cells have **antioxidant mechanisms**, which protect against potentially harmful oxygen-derived radicals (Table 1.2). These include:

- **Spontaneous decay**
- **Free radical-scavenging systems.**
 - Enzyme **catalase** neutralize peroxidase (H_2O_2) free radicals by converting it into water and oxygen.
 - Enzyme **superoxide dismutases (SODs)** neutralizes superoxide free radicals by converting it into hydrogen peroxide.
 - Enzyme **glutathione peroxidase** (enhances glutathione) neutralizes peroxidase (H_2O_2), hydroxyl and acetaminophen free radicals.
- **Exogenous antioxidants:** For example, vitamins E, vitamin A, ascorbic acid and glutathione.
- **Endogenous antioxidants:** Iron and copper are reactive metals, which can catalyze the formation of ROS. Their activities are minimized by binding of these ions to storage and transport proteins (e.g. transferrin, ferritin and ceruloplasmin).

Superoxide dismutase: Enzyme that protects the brain from free radical injury.

Fenton reaction leads to free radical generation when ferrous ions (Fe^{2+}) are converted to ferric ions.

TABLE 1.2: Various types of antioxidants

Enzymatic antioxidants	Non-enzymatic antioxidants
<ul style="list-style-type: none"> • Superoxide dismutase (SOD) • Catalase • Glutathione peroxidase 	<ul style="list-style-type: none"> • Exogenous: Vitamin E, vitamin A, ascorbic acid and sulfhydryl containing compounds (e.g. cysteine and glutathione) • Endogenous: Serum proteins, such as transferrin, ferritin, albumin and ceruloplasmin

Fenton reaction: $H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH + OH^-$

Pathologic Effects of Free Radicals (Fig. 1.11)

Free radicals can cause cell injury in many diseases. Free radicals can activate both **necrosis** and **apoptosis**. Various effects of free radicals are:

- **Lipid peroxidation in membranes** causes extensive membrane damage.
- **Cross-linking and oxidative modification of proteins** damages the enzyme activity and causes abnormal folding of proteins.
- **Damage to DNA.**

Free radicals steal electrons from neighboring molecules.

Free radicals can damage cellular membranes, proteins, and nuclear DNA.

Effects of Cell Injury

Defects in Membrane Permeability and Membrane Damage

- **Reversible injury:** In most forms of cell injury, in the early phase there is selective loss of membrane permeability.
- **Irreversible injury:** With the obvious membrane damage, the cell cannot return to normal.

Mechanisms of Membrane Damage

- **Indirect damage:**
 - **Reactive oxygen species (ROS):** It causes injury to cell membranes by **lipid peroxidation**.
 - **Decreased phospholipid synthesis:** Hypoxia through defective mitochondrial function → decreases the production of ATP by **ischemic cells** → leads to decreased phospholipid synthesis in all cellular membranes (including the mitochondria) and energy-dependent enzymatic activities.
 - **Increased phospholipid breakdown:** Severe cell injury increases levels of **cytosolic and mitochondrial Ca^{2+}** → results in **calcium-mediated activation** of endogenous **phospholipases** → which degrades membrane phospholipids → leads to the accumulation of **lipid breakdown products** → cause membrane damage.
 - **Cytoskeletal damage:** Cytoskeletal filaments connect the plasma membrane to the cell interior. **Increased cytosolic calcium activates proteases** which may damage the cytoskeletal elements and cell membrane.
- **Direct damage:** The plasma membrane can also be damaged directly by various bacterial toxins, viral proteins, lytic complement components and a variety of physical and chemical agents.

Consequences of Membrane Damage

Cell injury may damage any membrane, but most important are:

- **Mitochondrial membrane damage:** It results in:
 - Opening of the **mitochondrial permeability transition pore** leading to decreased ATP.
 - Release of proteins that trigger apoptotic death.
- **Plasma membrane damage:** It leads to **loss of**:
 - **Osmotic balance** and influx of fluids and ions
 - Cellular contents.
- **Lysosomal membrane damage:** It leads to:
 - **Leakage of lysosomal enzymes** into the cytoplasm
 - **Activation of lysosomal enzymes** → which results in digestion of proteins, RNA, DNA and glycogen → leads to cell death by necrosis.

Damage to DNA and Proteins

- **Causes of DNA damage:** Exposure to DNA damaging drugs, radiation or oxidative stress.
- **Repair mechanism:** Cells have mechanisms to repair DNA damage. However, if the damage is too severe to be corrected, the cell initiates a suicide program causing death by apoptosis.

ISCHEMIA-REPERFUSION INJURY

Q. Write short note on ischemia-reperfusion injury.

- **Decreased blood flow** to a tissue or organ is called **ischemia**.
- Depending on the severity and duration of ischemia, the involved tissue may adapt, undergo injury (reversible), or die (irreversible). Therapies to restore blood flow is an important modality of treating ischemia.
- If the involved cells of the tissue are reversibly injured, the restoration of blood flow (reperfusion) often beneficial. However, under certain circumstances the restoration of blood flow to cells that have been ischemic (reversibly injured) but have not died (irreversibly injured), can paradoxically exacerbate and produce injury at an accelerated pace.
- The damaging process is set in motion during reperfusion and **reperfused tissues undergoes loss of cells** (new damage) **in addition to the cells that are irreversibly damaged (died) at the end of ischemia**. This damaging process is called as **ischemia-reperfusion injury**.
- **Clinical importance:** It contributes to tissue damage following reperfusion in **myocardial infarction** and **cerebral infarction**.

Mechanism of Reperfusion Injury

Free radicals in reperfusion injury are mainly produced by infiltrating leukocytes.

New damage may be initiated during reoxygenation includes:

1. **Increased generation of reactive oxygen and nitrogen species:**
 - **Increased production of free radicals:** They may be produced from parenchymal and endothelial cells and from infiltrating leukocytes in reperfused tissue as a result of mitochondrial damage, causing incomplete reduction of oxygen, or because of the action of oxidases in leukocytes, endothelial cells, or parenchymal cells.
 - **Decreased antioxidant mechanism:** Ischemia may result in defective cellular antioxidant defense mechanisms, favoring the accumulation of free radicals.
2. **Inflammation:** Ischemic injury produces cytokines and increased expression of adhesion molecules by hypoxic parenchymal and endothelial cells. They recruit circulating neutrophils to reperfused tissue causing inflammation. The inflammation causes further tissue injury.
3. **Activation of the complement system:** It is an important mechanism of immune-mediated injury. Some IgM antibodies may get deposited in ischemic tissues. When blood flow is restored, complement proteins may bind to the deposited antibodies and complement system may be activated → cause inflammation and more injury to cells.

TYPES OF CELL INJURY

Two types: **Reversible and irreversible**. Reversible injury may progress to a reversible stage and result in cell death.

Reversible Cell Injury

If the stimulus is acute and brief or mild, the cell injury produces changes in the cells which are reversible up to a certain point.

Light microscope features of reversible cell injury: Two patterns of reversible cell injury namely cellular swelling and fatty change.

- **Cellular (hydropic) swelling:** It is due to changes in ion concentrations and fluid homeostasis. There is increased flow of water into the cells and results in increased water content of injured cells.
- **Steatosis (fatty change)** explained above.

Steatosis (Fatty Change)

Q. Write short note on causes, pathogenesis and morphology of fatty/steatosis liver. Add a note on special stains for fat.

Abnormal accumulations of triglycerides within cytosol of the parenchymal cells.

Organs involved: Seen in organs involved in fat metabolism namely **liver**. It may also occur in heart, muscle and kidney.

Causes

- **Disorders with hepatocyte damage:** Alcoholic abuse, protein malnutrition, starvation, anoxia (anemia, cardiac failure), toxins (carbon tetrachloride, chloroform, etc.) and Reye syndrome. **Alcohol is the most common cause** of fatty change in the liver.
- **Disorders with hyperlipidemia:** Obesity, diabetes mellitus or congenital hyperlipidemia.

Pathogenesis of Fatty Liver

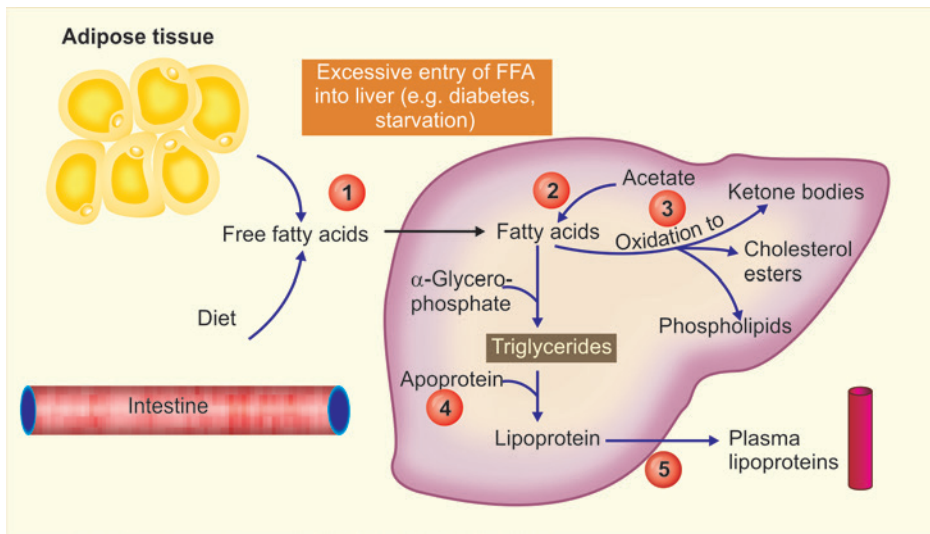
Various mechanisms are involved in excess accumulation of triglyceride in the liver and one or more mechanism may be responsible.

- **Excessive entry of free fatty acids (FFA) into the liver** (1 in Fig. 1.13): From peripheral stores FFA enters into liver during starvation and diabetes.
- **Defective metabolism of lipids:** This may be due to:
 - Increased synthesis of fatty acids by liver (2 in Fig. 1.13).
 - Decreased oxidation of fatty acids into ketone bodies (3 in Fig. 1.13) resulting in increased esterification of fatty acids into triglycerides.
 - Decreased synthesis of apoproteins (e.g. in **CCl₄ and protein malnutrition**) causes decreased formation of lipoproteins from triglycerides (4 in Fig. 1.13).
- **Defective excretion of lipoproteins:** Fatty liver may also develop due to defect in excretion of lipoproteins from liver into the blood (5 in Fig. 1.13).

MORPHOLOGY

Fatty Liver

- **Gross** (Fig. 1.14): Liver enlarges and becomes **yellow, soft and greasy to touch**.
- **Microscopy** (Figs 1.15 and 19.15): First, fat is seen as small **vacuoles in the cytoplasm** around the nucleus. Later, the vacuoles coalesce, creating clear spaces that displace the nucleus to the periphery of the cell.
- **Special stains for fat:** Frozen sections stained with Sudan IV or Oil Red-O give an orange-red color to the fat. Osmic acid gives a black color.



Starvation: Increases fatty acid mobilization from peripheral stores.

Steatosis of liver may be due to:
 1. Excessive entry free fatty acids
 2. Defective metabolism of lipids
 3. Defective export of lipoproteins.

Alcohol is the most common cause of steatosis of liver.

Hypoxia inhibits fatty acid oxidation.

Fig. 1.13: Various mechanisms that can produce accumulation of triglycerides in fatty liver

Heart

Q. Write short note on heart in fatty change.

Lipid in the cardiac muscle can have two patterns:

- **Alternate involvement:** Prolonged moderate hypoxia (e.g. severe anemia), create grossly apparent bands of involved yellow myocardium alternating with bands of darker, red-brown, uninvolved myocardium (**tigered effect, tabby cat appearance**).
- **Uniform involvement:** More severe hypoxia or some types of myocarditis (e.g. diphtheria infection) show more uniform involvement of myocardial fibers.



Fig. 1.14: Fatty liver showing a part of liver with yellow color and sharp border

Cholesterol Deposits

Intracellular accumulation of cholesterol or cholesterol esters in macrophages may occur when there is hypercholesterolemia. It appears microscopically as intracellular.

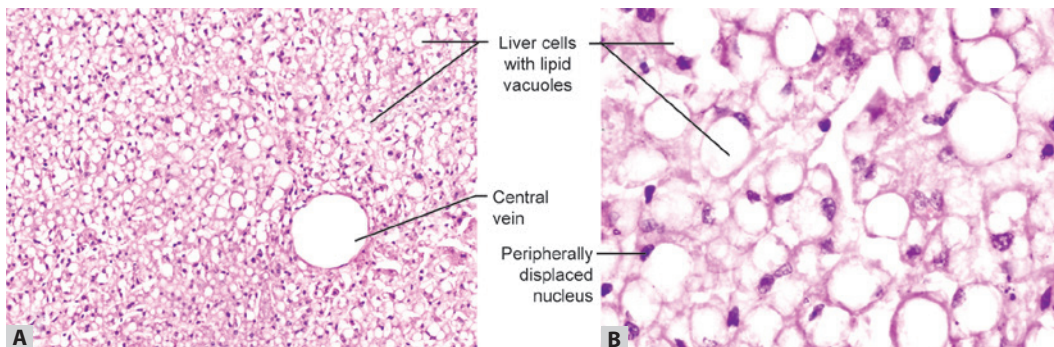
Atherosclerosis

It is a disease of **aorta and large arteries** characterized by the presence of **atherosclerotic plaques composed of smooth muscle cells and macrophages within the intima filled with lipid vacuoles**. Most of the lipid is **cholesterol and cholesterol esters** (refer Chapter 14).

Xanthoma

Q. Write short note on xanthoma.

Intracellular accumulation of cholesterol within macrophages is found in acquired and hereditary hyperlipidemic



Figs 1.15A and B: (A) Fatty liver in which the hepatocytes show accumulation of fat which appear as clear vacuole in the cytoplasm; (B) Hepatocytes at higher magnification in which the nucleus is displaced to the periphery by accumulated fat

states. The tumor mass produced by the macrophages filled with cholesterol is termed xanthomas. Microscopically, it consists of clusters of foamy cells in the subepithelial connective tissue of the skin and in tendons.

Irreversible Cell Injury

If the cell is exposed to continuous injurious stimulus or if the injury is severe, the cells undergo cell death. Two main types of cell death: **Necrosis and apoptosis**.

- **Necrosis:** Always a pathologic process (refer below).
- **Apoptosis:** May be physiological or pathological (refer page 22).

NECROSIS

Q. Define necrosis. Describe the various types of necrosis, causes and pathology of each with suitable examples.

Definition: Morphological changes indicative of cell death in a living tissue following harmful injury. Necrosis is an “accidental” and unregulated form of cell death. It results from damage to cell membranes and loss of ion homeostasis. The necrotic cells cannot maintain integrity of membrane and their contents leak out. This brings out acute **inflammatory reaction** in the surrounding tissue.

MORPHOLOGY (FIG. 1.16)

The general changes occurring in a necrotic cell:

- **Cytoplasmic changes:** Increased eosinophilia.
- **Nuclear changes:** These may take up one of three patterns:
 - **Pyknosis:** Shrinkage of nucleus which appears shrunken and deeply basophilic (similar to ink drop).
 - **Karyolysis:** Progressive fading of basophilic staining of the nuclei and leads to a ghost nuclei.
 - **Karyorrhexis:** Nucleus breaks up into many smaller fragments.

Electron microscopic findings of necrosis are diagrammatically shown in Figure 1.16.

Patterns/Types of Tissue Necrosis

Coagulative Necrosis

Q. Write short note on coagulative necrosis.

Common type, outline of dead tissues is preserved (at least for few days). **Infarct** is a localized area of coagulative necrosis.

- **Causes:** Ischemia caused by obstruction in a vessel.
- **Mechanism:** Ischemia denatures and coagulates structural proteins and enzymes.

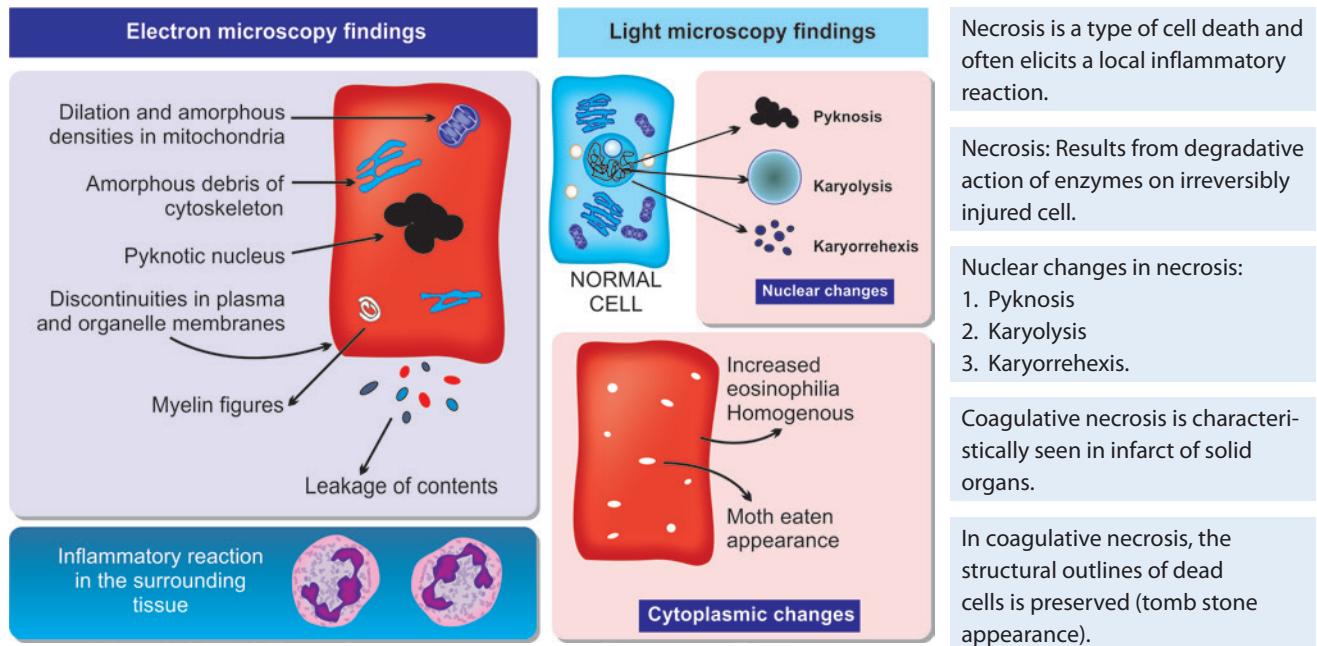
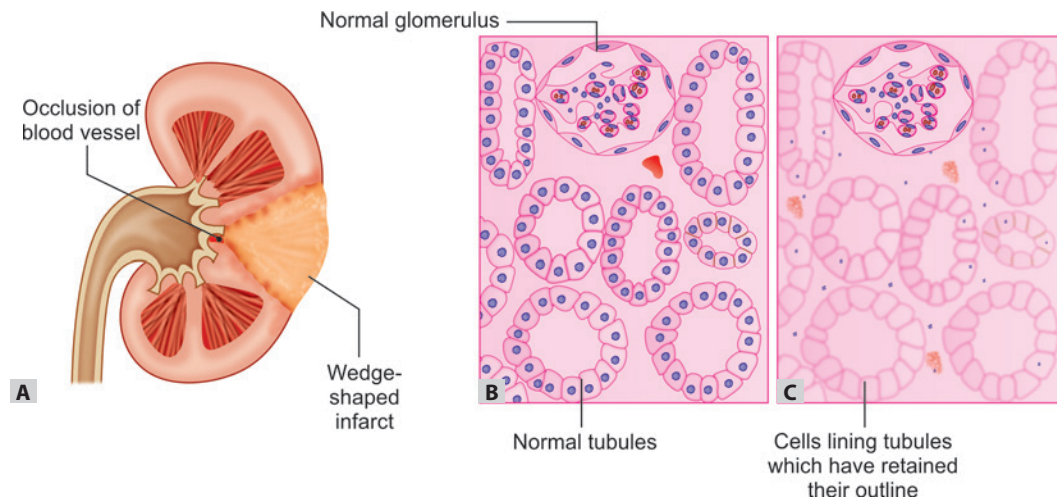
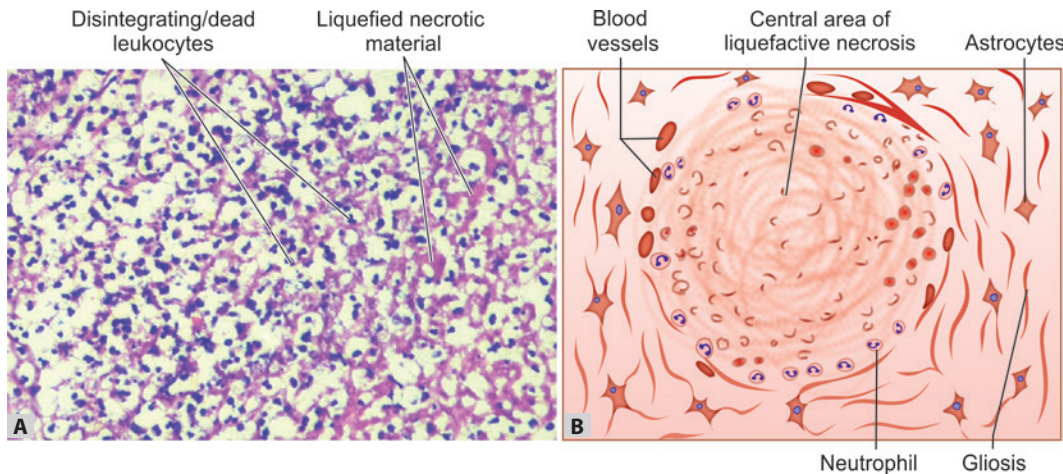


Fig. 1.16: Morphological changes in necrosis



Figs 1.17A to C: (A) Gross appearance of infarct of kidney; (B) Microscopy of normal kidney parenchyma; (C) Infarcted area of kidney



Figs 1.18A and B: Microscopic appearance of an abscess consisting of liquified necrotic cell debris and dead/disintegrating neutrophils. (A) Hematoxylin and eosin; (B) Diagrammatic appearance of brain abscess

Fate of necrotic cell:

- Digestion by enzymes
- Replacement by myelin figures
- Calcification.

Ischemic injury to CNS cause liquefactive necrosis and NOT coagulative necrosis.

Liquefactive necrosis: Characteristically seen in ischemic injury to CNS and suppurative/pyogenic infections.

• **Gross:**

- **Organs affected:** All organs except the brain. More frequent in heart, kidney, spleen and limb (dry gangrene).
- **Appearance:** Involved region appear dry, pale, yellow and firm. It is wedge-shaped in organs like kidney (Fig. 1.17A) and spleen.

- **Microscopy** (Figs 1.17B and C and refer Figs 15.5 and 21.36): Indistinct outline of dead tissue. Nucleus may be either absent or show karyolysis.

Dead tissue rapidly undergoes softening and transforms into a liquid viscous mass.

• **Causes:**

- **Ischemic injury to central nervous system (CNS)**
- **Suppurative infections:** Infections by bacteria which stimulate the accumulation of leukocytes.
- **Mechanism:** Liquefaction is due to digestive action of the hydrolytic enzymes released from dead cells (autolysis) and leukocytes (heterolysis).

Liquefactive Necrosis (Colliquative Necrosis)

Q. Write short note on liquefactive/colliquative necrosis.

Liquefactive necrosis: Dead cells are transformed into a liquid viscous mass due to enzymes released from leukocytes accumulated at the site of necrosis.

• **Gross:** Organs affected are:

- **Brain:** Necrotic area is soft and center show liquefaction.
- **Abscess anywhere:** Localized collection of pus.
- It is also seen in wet gangrene and pancreatic necrosis.

- **Microscopy** (Fig. 1.18): Pus consists of liquefied necrotic cell debris, dead leukocytes and macrophages (scavenger cells).

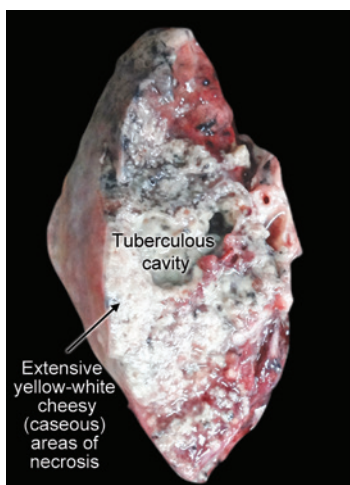


Fig. 1.19: Gross appearance of caseous necrosis. Lung shows cavity with caseous necrosis

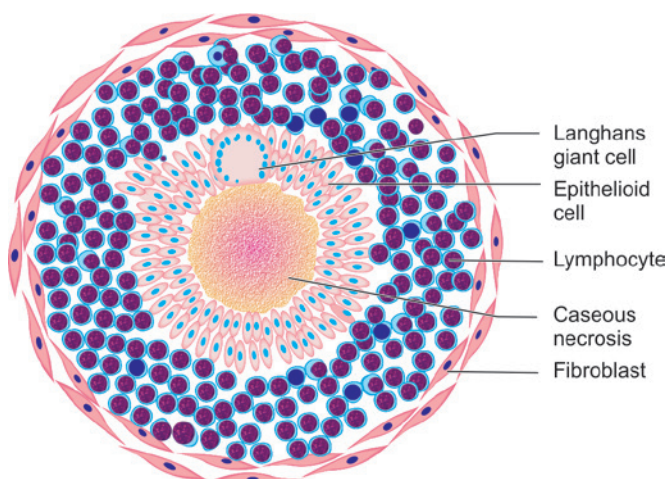


Fig. 1.20: Microscopic (diagrammatic) appearance of a caseous necrosis

Caseous Necrosis

Q. Write short note on caseous necrosis.

Caseous necrosis: Cheese-like appearance of the necrotic material.

Caseous necrosis with granuloma is observed in tuberculosis and systemic fungal infections (e.g. histoplasmosis). It is due to the presence of high lipid content in the cell wall in these organisms.

Distinctive type of necrosis which shows **combined features of both coagulative and liquefactive necrosis**.

- **Cause:** Characteristic of **tuberculosis** and is due to the hypersensitivity reaction.
- **Gross:**
 - **Organs affected:** Tuberculosis may involve any organ, most common in **lung** and **lymph node**.
 - **Appearance:** Necrotic area appears **yellowish-white, soft, granular** and resembles **dry, clumpy cheese**, hence the name caseous (**cheese-like**) necrosis (Figs 1.19 and 16.20).
- **Microscopy:**
 - Focal lesion of tuberculosis is a **granuloma** (Figs 1.20, 4.1 and 16.19) which may be **caseating (soft granuloma)** or **noncaseating (hard granuloma)**.
 - ◆ Caseous necrosis appears as **eosinophilic, coarsely granular material**. It is surrounded by epithelioid cells; Langhans type giant cells (nuclei arranged in a horse-shoe pattern), lymphocytes and fibroblasts.
 - ◆ Caseous necrotic material **may undergo dystrophic calcification**.

Fat Necrosis

Q. Write short note on fat necrosis.

It refers to **focal areas of fat destruction**, which affects adipose tissue.

Types:

1. **Enzymatic fat necrosis:** Occurs in adipose tissue around acutely inflamed pancreas (in **acute pancreatitis**).
 - **Mechanism:** In pancreatitis, the enzymes (one of them is lipase) leak from acinar cells and causes tissue damage. Lipase destroys fat cells and liberates free fatty acids which combine with calcium and form calcium soaps (fat saponification).
2. **Traumatic fat necrosis:** Occurs in tissues with high fat content (like in **breast and thigh**) following severe trauma.

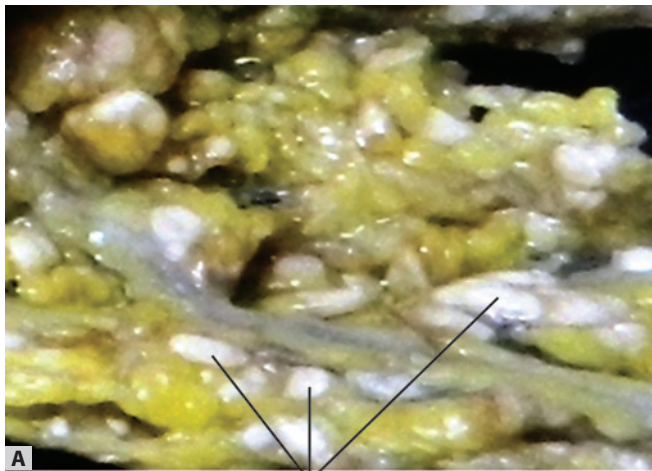
- **Gross:** Appears as **chalky-white areas** (Fig. 1.21A).
- **Microscopy:** The necrotic **fat cells appear pale with shadowy outlines** surrounded by an inflammatory reaction (Fig. 1.21B).

Enzymatic fat necrosis is mediated by enzymes whereas traumatic is not mediated by enzymes.

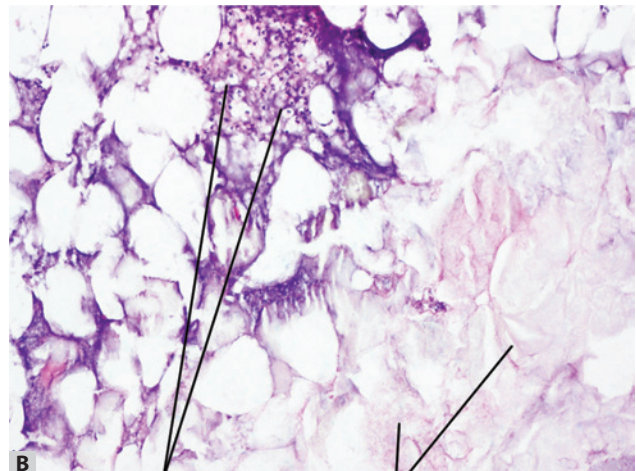
Enzymatic fat necrosis in acute pancreatitis appears as chalky white areas which help in its gross identification.

Fibrinoid Necrosis

Characterized by **deposition of pink-staining (fibrin-like) proteinaceous material** in the tissue matrix with a **staining**



Omentum with multiple chalky white areas of fat necrosis



Inflammatory cells Fat cells with shadowy outline and opaque cytoplasm

Figs 1.21A and B: (A) Omentum shows multiple chalky white areas of fat necrosis caused by acute pancreatitis; (B) Fat necrosis shows necrotic fat cells in the right lower part and inflammatory reaction between normal (left upper area) and area of fat necrosis

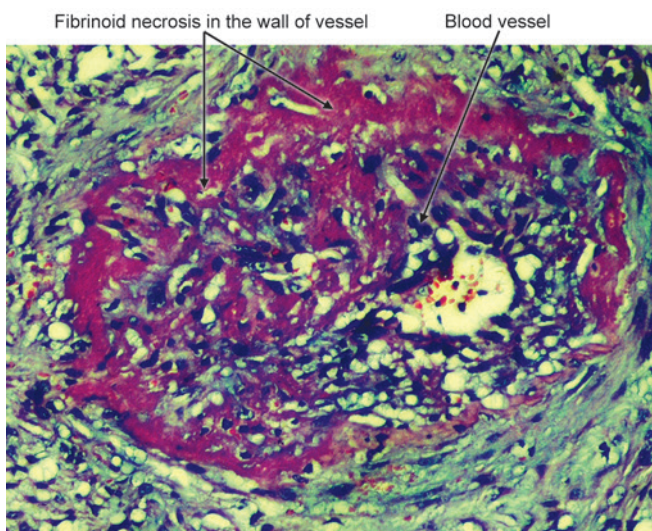


Fig. 1.22: Fibrinoid necrosis in the wall of blood vessel

pattern reminiscent of fibrin (Figs 1.22 and 14.10). It obscures the underlying cellular detail.

- **Causes:** Usually seen in **immune-mediated** (deposition of antigen-antibody complexes in the wall of vessels) **vascular injury/vasculitis** (e.g. polyarteritis nodosa), **malignant hypertension**, **Aschoff bodies** in rheumatic heart disease, placenta in **preeclampsia**, or **hyperacute transplant rejection**.

Fibrinoid necrosis: Necrotic material appears similar to fibrin and is not fibrin.

Fibrinoid necrosis: Seen in immune-mediated diseases

1. Polyarteritis nodosa
2. Malignant hypertension
3. Autoimmune disorder—SLE
4. Aschoff bodies in rheumatic fever.

Gangrene (Gangrenous Necrosis)

Q. Define gangrene. Mention its types and differences between them.

It is massive necrosis with superadded putrefaction.

Types: Two types, namely **dry** and **wet gangrene**. A variant of wet gangrene known as **gas gangrene** is caused by **clostridia** (Gram-positive anaerobic bacteria).

Dry Gangrene

- **Causes:** **Arterial occlusion** (e.g. atherosclerosis).
- **Sites:** It usually involves a **limb**, generally the distal part of lower limb (leg, foot, and toe).

- **Gross:** Affected part is **dry, shrunken** (shriveled) and **dark brown or black** resembling the foot of a **mummy**. The black color is due to the iron sulfide. A **line of demarcation** is seen between gangrenous and adjacent normal area (Fig. 1.23).
- **Microscopy:** The necrosis (coagulative type) shows smudging of soft tissue and overlying skin. The line of demarcation consists of granulation tissue with inflammatory cells.

Dry gangrene predominantly consists of coagulative type of necrosis.

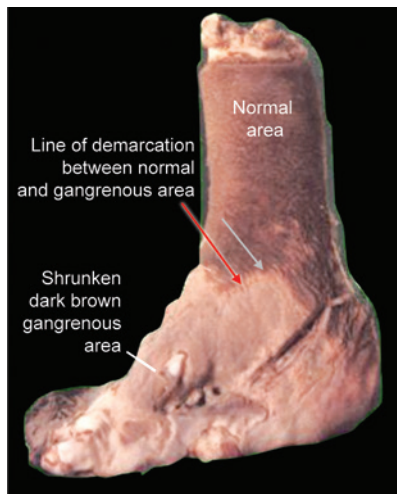


Fig. 1.23: Dry gangrene of left leg shows dry shrunken discolored gangrenous foot separated from adjacent normal area by a line of demarcation

Wet Gangrene

- **Causes:** Due to the venous blockage (e.g. strangulated hernia, intussusception or volvulus).
- **Sites:** Occurs in moist tissues or organs (e.g. bowel, lung, mouth, etc.).
- **Gross:** The affected part is soft, swollen, putrid and dark. No clear line of demarcation.
- **Microscopy:** Liquefactive type of necrosis.

Wet gangrene predominantly consists of liquefactive type of necrosis.

Fournier's gangrene: Seen in scrotal skin

Differences Between Dry and Wet Gangrene (Table 1.3)

Q. List the differences between dry and wet gangrene.

Gas gangrene: Special type of wet gangrene caused by infection with a gas forming anaerobic clostridia. These organisms enter into the tissues through open contaminated wounds (e.g. muscles, complication of operative procedures on colon). Toxins produced by them cause local necrosis and edema and are also absorbed causing severe systemic manifestations.

Gas gangrene is a variant of wet gangrene caused by clostridia (Gram-positive anaerobic bacteria).

Gummatous Necrosis

The necrotic tissue is firm and rubbery and is usually found in syphilis.

TABLE 1.3: Differences between dry and wet gangrene

Characteristics	Dry	Wet
General features		
Common site	Limbs	Bowels
Examples	Gangrene due to atherosclerotic narrowing of blood vessel of lower limb	Volvulus, intussusception
Etiological factors		
Cause of ischemia	Arterial obstruction	Commonly venous obstruction
Rate of obstruction	Slow	Abrupt
Gross features		
Appearance of involved part	Shriveled dry (mummification) and black	Swollen, soft and moist
Line of demarcation	Clear cut	Not clear cut
Spread	Slow	Rapid
Prognosis	Fair	Poor due to severe septicemia

Ultrastructural differences between reversible and irreversible injury is presented in Table 1.4.

APOPTOSIS

Q. Write short note on apoptosis.

Apoptosis: Affects only single or small group of cells.

Apoptosis is a **type of (programmed) cell death induced by a tightly regulated suicide program**. It is characterized by **activation of intrinsic enzymes of the cell that degrade its own nuclear DNA and proteins** (nuclear and cytoplasmic).

Causes of Apoptosis

Apoptosis may be physiological or pathological.

Physiological Situations

- **Removal of excess cells during embryogenesis and developmental processes:** For example, disappearance of web tissues between fingers and toes.
- **Elimination of cells after withdrawal of hormonal stimuli:** For example, endometrial cell breakdown during the menstrual cycle.

TABLE 1.4: Ultrastructural differences between reversible and irreversible injury**Q. Describe the ultrastructural changes in reversible cell injury.**

Structure involved	Reversible injury	Irreversible injury
Plasma membrane changes	Blebbing, blunting, loss of microvilli	Discontinuities in plasma and organelle membrane
Mitochondrial changes	Swelling and appearance of small amorphous densities	Marked dilatation with appearance of large amorphous densities (precipitated calcium), aggregates of fluffy material (denatures protein)
Endoplasmic reticulum	Dilatation with detachment of polysomes	Swelling and fragmentation
Myelin figure (large intracellular whorled phospholipid masses)	May be present	Usually present
Nuclear changes	Disaggregation of granular and fibrillar elements	Pyknosis, karyolysis and karyorrhexis

Irreversible injury: Large amorphous densities in mitochondria.

- **Elimination of cells after withdrawal of tropic stimuli:** For example, neutrophils in an acute inflammatory response, lymphocytes after immune response.
- **Elimination of potentially harmful cells:** In immunology, the clones of **self-reactive lymphocytes** that recognize normal self antigens are deleted by apoptosis.

Pathological Conditions

Apoptosis eliminates cells that are genetically altered or damaged beyond repair. It is responsible for cell loss in many pathologic states:

- **Elimination of cells with damaged DNA:** DNA may be damaged by many injurious agents like radiation, cytotoxic anticancer drugs and hypoxia.
 - Mainly tumor-suppressor gene p53 recognizes cells with damaged DNA and assesses whether it can be repaired. If the damage is too severe to be repaired, p53 triggers apoptosis.
 - **Destroying cells with dangerous mutations or with DNA damage** beyond repair by apoptosis prevents the development of cancer.
 - In certain cancers, where p53 is mutated or absent, the apoptosis is not induced in cells with damaged DNA.
- **Elimination of cells with excessively accumulated misfolded proteins:** Mutations in the genes encoding proteins or extrinsic factors (damage due to free radicals) may result in accumulation of unfolded or misfolded proteins.
 - Excessive intracellular accumulation of these abnormally folded proteins in the ER is known as **ER stress**, which results in apoptotic cell death.

- Apoptosis caused by the accumulation of misfolded proteins is found in several degenerative diseases of the central nervous system (Alzheimer, Huntington, and Parkinson diseases) and other organs.
- **Killing of viral infected cells:** In viral infections, the infected cells are lost mainly due to apoptosis induced either by the virus (as in adenovirus and HIV infections) or by host human response by cytotoxic T lymphocytes (as in viral hepatitis).
- **Elimination of neoplastic cells/rejection of transplant:** The T-cell-mediated mechanism is responsible for apoptosis in tumors and cellular rejection of transplants.
- **Elimination of parenchymal cells in pathologic atrophy:** Obstruction of duct in the **parenchymal** organs like pancreas, parotid gland and kidney can lead to apoptosis of the parenchymal cells.

MORPHOLOGY

Electron Microscope

Q. Write short note on morphology of apoptosis.

The ultrastructural features of apoptosis (Fig. 1.24) are:

- **Cell shrinkage:** Cytoplasm becomes dense.
- **Nuclear condensation and fragmentation:** Chromatin aggregates peripherally under the nuclear membrane. The nucleus may break up to produce two or more nuclear fragments.
- **Formation of cytoplasmic blebs and apoptotic bodies:** Cell first shows extensive surface blebbing followed by fragmentation into membrane-bound apoptotic bodies. The apoptotic bodies are composed of cytoplasm and tightly packed organelles, with or without nuclear fragments.
- **Phagocytosis of apoptotic cells/bodies:** The apoptotic bodies are rapidly ingested by phagocytes (**usually by macrophages**) and degraded by the lysosomal enzymes of phagocytes.

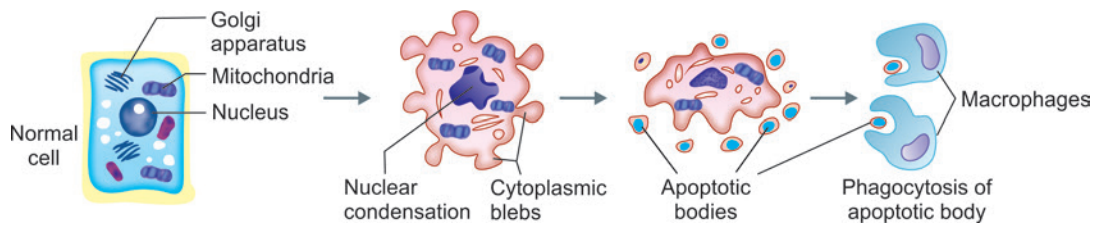


Fig. 1.24: Electron microscopic changes in apoptosis

Light Microscopy

Light microscopic characteristics of apoptosis:

- Condensation of nucleus (pyknotic)
- Deeply eosinophilic cytoplasm.

The apoptotic cells appear as round or oval mass having intensely eosinophilic cytoplasm. The nuclei appear as fragments of dense nuclear chromatin and shows pyknosis. Apoptosis does not elicit an inflammatory reaction in the host.

Mechanisms of Apoptosis

Q. Write short note on mechanism of apoptosis.

The survival or apoptosis of many cells depends upon balance between two opposite sets of signals namely (1) **death signal** (proapoptotic) and (2) **prosurvival** (anti-apoptotic) signals. Unlike necrosis, apoptosis engages the cell's own signaling cascades and results in its own death (suicide). Apoptosis results from **activation of enzymes** called as **caspases** (i.e. they are cysteine proteases that cleave proteins after aspartic residues).

Phases of Apoptosis

Divided into (A) initiation phase and (B) execution phase.

A. Initiation phase

Apoptosis: Organelle that plays a pivotal role is mitochondria.

Apoptosis is initiated by signals derived from two distinct pathways activated by distinct stimuli, namely (1) intrinsic or mitochondrial pathway and (2) extrinsic or death receptor pathway.

1. Intrinsic (mitochondrial) pathway of apoptosis (Fig. 1.25): It is activated by intracellular signals.

• Role of mitochondria in apoptosis:

- Mitochondrial damage is the major mechanism in a variety of physiological and pathological apoptosis.
- Mitochondria contain proteins capable of inducing apoptosis. These include: **cytochrome c** and several proapoptotic proteins.

- Survival or apoptosis of cell is determined by permeability of mitochondria.
- **Mitochondrial permeability is controlled by BCL2 family** of more than 20 proteins. This family is named after *BCL2*, which was identified as an oncogene in a **B-cell** lymphoma. These proteins may be broadly divided into proapoptotic or anti-apoptotic (prosurvival).

♦ Proapoptotic proteins: BAX and BAK

♦ Antiapoptotic proteins: BCL2, BCL-XL, and MCL1. They prevent leakage of mitochondrial proteins that trigger apoptosis. Growth factors and other survival signals stimulate production of antiapoptotic proteins.

If the balance shifts to proapoptotic proteins, the apoptotic cascade is activated.

• Causes of mitochondrial injury: The proapoptotic signals include:

- Deprivation/withdrawal of growth factor or survival signals.
- DNA damage by radiation, cytotoxic anticancer drugs, hypoxia either directly or through free radical.
- Accumulation of excessive amount of misfolded proteins (endoplasmic reticulum stress).
- Increased intracellular free calcium.

• Steps in intrinsic (mitochondrial) pathway: Mitochondrial injury causes increased mitochondrial permeability and release proapoptotic molecules (death inducers) into the cytoplasm. The different steps are as follows:

- The above mentioned causes of mitochondrial injury activate a number of sensors of BCL2 family called BH3-proteins. They in turn activate two critical proapoptotic BCL2 family effector proteins, namely **BAX** and **BAK**.
- BAX and BAK create channels in the mitochondria that allow release of several mitochondrial proteins from the inner mitochondrial membrane to leak out into the cytosol (cytoplasm).
- One of these proteins is **cytochrome c** which binds to a protein called **apoptosis-activating factor-1**

(Apaf-1) and forms an important caspase cascade activator called **apoptosome**. This complex binds to caspase-9, the critical initiator caspase of the mitochondrial pathway which sets in an auto-amplification process.

- The cytoplasm of the normal cells contains proteins which block the activation of caspases and function as physiologic **inhibitors of apoptosis (called IAPs)**. Other mitochondrial proteins may enter the cytoplasm and **neutralize** these IAPs and initiate caspase cascade.
- Sensors of **BCL2 family** namely BH3-only proteins also bind to and **block the function of protective antiapoptotic proteins** namely **BCL2** and **BCL-XL**.

- Glucocorticoids induce apoptosis
- Sex steroids inhibit apoptosis.

BCL2 family genes located on chromosome 18.

Apoptosis: Apaf-1 is activated by the release of cytochrome c from mitochondria.

2. Extrinsic (death receptor-initiated) pathway of apoptosis (Fig. 1.25)

- This pathway is **initiated by extracellular signals**.
- Many cells express “death-receptors” molecules on the surface of plasma membrane that trigger apoptosis. Death receptors are member of the TNF (tumor necrosis factor) receptor family that contain a cytoplasmic domain called the **death domain** because it is essential for delivering apoptotic signals.
- In the extrinsic (death receptor) pathway, **apoptosis is initiated when the death receptors present gets activated**.
- The well-known death receptors are the **type 1 TNF receptor (TNFR1)** and a related protein called **Fas (CD95)**. Fas death receptor is expressed on many cell types and the binding ligand for Fas is called Fas ligand (FasL/CD95L).
- **Functions of extrinsic pathway:** This pathway is involved in eliminating:
 - **Self-reactive lymphocytes** thereby avoiding autoimmunity. FasL is expressed on T-cells that recognize self-antigens and function to eliminate self-reactive lymphocytes.
 - **Virus infected cells** through cytotoxic T lymphocytes.
 - **Tumor cells** through cytotoxic T lymphocytes.

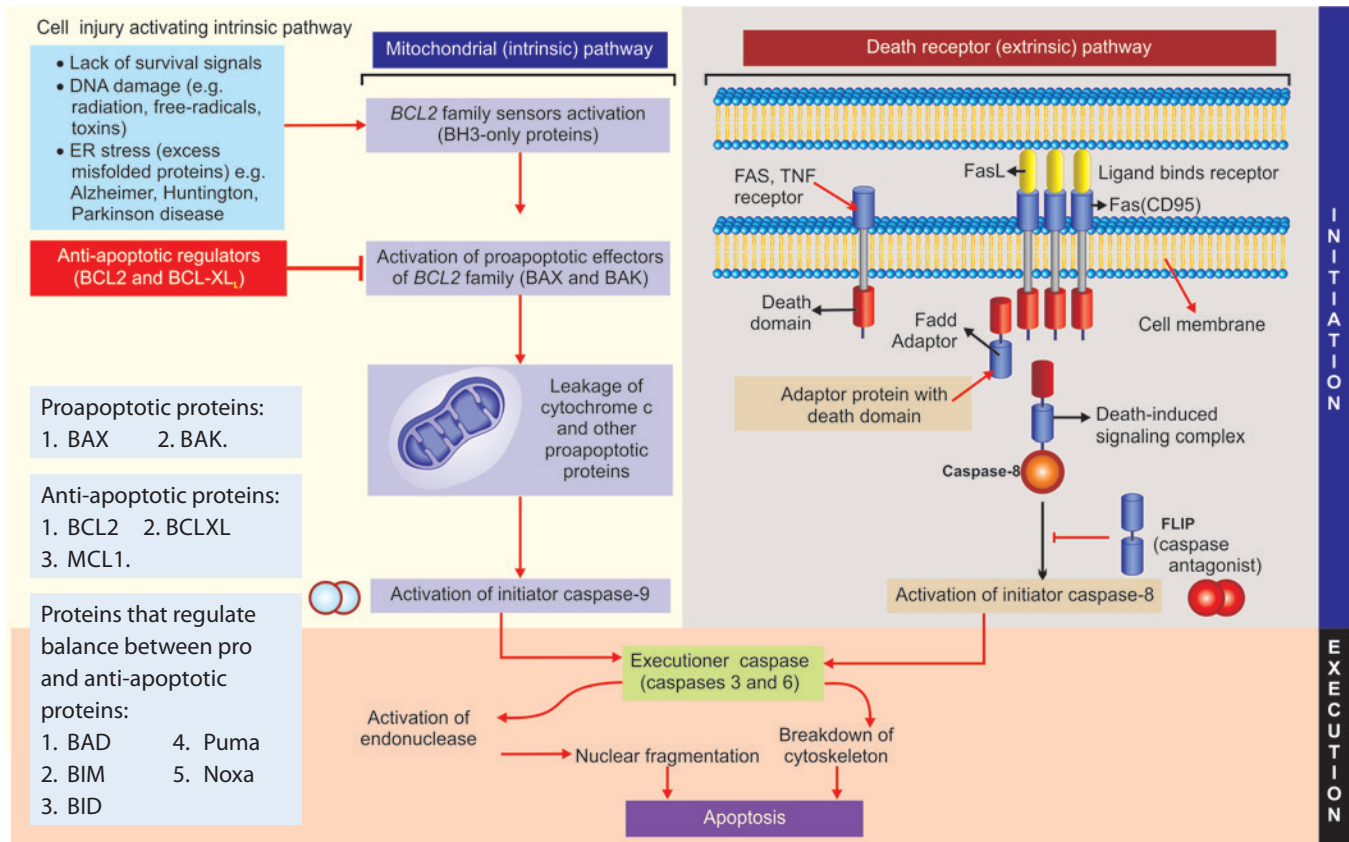


Fig. 1.25: Mechanism of apoptosis

CD 95 (FAS) has a major role in apoptosis and is molecular marker for apoptosis.

Apoptosis: Extrinsic pathway through TNFR1.

- **Steps in extrinsic pathway:**
 - Extrinsic pathway become activated when CD95/ Fas binds to its ligand CD95L/FasL.
 - When FasL binds to Fas receptors, their cytoplasmic death domains binds with an **adapter protein**. This adapter protein also contains a death domain and is called Fas-associated death domain (FADD).
 - FADD in turn binds to pro-caspase-8 (an inactive form of caspase-8) via a death domain and generate active caspase-8.
 - Activated caspase-8 mediate the execution phase of apoptosis.

B. Execution Phase of Apoptosis (Fig. 1.25)

- The above mentioned two initiating pathways produce initiator caspases namely: (1) the **mitochondrial pathway activates initiator caspase-9**, and (2) the **death receptor pathway activates the initiator caspase-8**.
- The initiator caspases activate another series of caspases called **executioner caspases (such as caspase-3 and -6)** that mediates the final phase of apoptosis.
- Executioner caspases act on many cellular components and activate DNase, which induces fragmentation of nuclei.
- Caspases also degrade components of nuclear matrix and cytoskeleton resulting in fragmentation of involved cells.

Caspases: Initiators and executioners.

Mechanism of apoptosis has two major steps namely initiation and execution.

Apoptosis is mediated by caspases.

Removal of Apoptotic Cells

Apoptosis is a regulated mechanism of cell death with the least possible reaction by host.

- **Phagocytosis:** Apoptotic cells and bodies are engulfed and removed by phagocytic cells (mainly macrophages). The phagocytosis is so efficient that these dead cells and apoptotic bodies disappear within minutes. Even when the apoptosis is extensive their rapid removal prevents release of their cellular contents which may elicit inflammation.

- **Factors favoring phagocytosis:** The apoptotic cells and apoptotic bodies undergo several changes in their membranes and produce signals that favor phagocytosis of these cells/bodies.

- **Expression of phosphatidylserine:** In healthy cells, **phosphatidylserine** is present on the inner leaflet of the plasma membrane. In cells undergoing apoptosis **phosphatidylserine** turns out and is expressed on the outer layer of the membrane causing easy recognition by receptors present on the macrophage.
- **Secretion of soluble factors:** **Apoptotic cells secrete soluble factors** (e.g. **thrombospondin**) that recruit phagocytes.
- **Natural antibodies and proteins of the complement system** may coat apoptotic bodies which aids in phagocytosis.

Diagnosis/Detection of Apoptosis

1. DNA fragmentation assay is carried out by electrophoresis of genomic DNA. Apoptosis produces “step ladder pattern” in contrast to smeared pattern seen in necrosis.
2. Terminal deoxynucleotidyl transferase biotin d-UTP Nick End Labeling (TUNEL) technique for in vivo detection of apoptosis.
3. Chromatin condensation seen by hematoxylin and eosin, Feulgen and acridine orange staining.
4. Estimation of:
 - Cytosolic cytochrome c
 - Activated caspase
 - Annexin V: Apoptotic cells express phosphatidylserine on the outer layer of plasma membrane because of which these cells are recognized by the dye Annexin
 - Propidium iodide assay by flow cytometry/fluorescent microscopy.

Annexin V on non-permeable cell is indicative of apoptosis. Annexin attaches to cell surface.

Apoptosis: Ladder pattern of DNA electrophoresis is caused by enzyme endonuclease.

Disorders Associated with Dysregulated Apoptosis

- **Disorders with reduced apoptosis:** It may allow the survival of abnormal cells.
 - Cancer
 - Autoimmune disease.

TABLE 1.5: Differences between apoptosis and necrosis

Features	Apoptosis	Necrosis
Cause	Often physiological, means of eliminating unwanted cells; may also be pathological	Invariably pathological
Biochemical events	Energy-dependent fragmentation of DNA by endogenous endonucleases	Impairment or cessation of ion homeostasis
Lysosomes	Intact	Leak lytic enzymes
Morphology		
Extent	Single or small cluster of cells	Involves group of cells
Cell size	Cell reduced (shrinkage) and fragmentation to form apoptotic bodies with dense chromatin	Cell enlarged (swelling) and undergo lysis
Integrity of cell membrane	Maintained	Disrupted/lost
Nucleus	Fragmentation into nucleosome-size fragments	Pyknosis, karyorrhexis, karyolysis
Cellular contents	Intact; may be released in apoptotic bodies	Enzymatic digestion; may leak out of cell
Adjacent Inflammatory response	None	Usual
Fate of dead cells	Ingested (phagocytosed) by neighboring cells	Ingested (phagocytosed) by neutrophil polymorphs and macrophages
DNA electrophoresis	DNA laddering is seen	Shows smearing effect
TUNEL staining	Positive	Negative

Apoptosis : No inflammatory response from adjacent tissue.

Leakage of proteins from the necrotic cells into the circulation is useful for identifying the necrosis using blood and serum samples.

- **Disorders with increased apoptosis:** This will cause an excessive loss of cells.
 - **Neurodegenerative diseases (Alzheimer, Huntington, Parkinson disease).**
 - **Ischemic injury:** In myocardial infarction and stroke.
 - **Death of virus-infected cells:** Many viral infections, important being acquired immune deficiency syndrome (AIDS).

Clinical Significance of Apoptosis in Cancers

- Normally, cells with damaged (mutated) DNA are cleared in the body by undergoing apoptosis.
- Apoptosis may be reduced in some cancers. Best established role of BCL2 in protecting tumor cells from undergoing apoptosis is observed in **follicular lymphoma**. In this type of non-Hodgkin lymphoma of B cell origin, there is **translocation (14; 18) (q32; q21)** which causes **over expression of antiapoptotic protein BCL2**. This in turn increases the BCL2/BCL-XL buffer, **protecting abnormal B lymphocytes from undergoing apoptosis** and allows them to survive for long periods.

Q. List the differences between apoptosis and necrosis.

Differences between apoptosis and necrosis are summarized in Table 1.5.

- **Necroptosis:** It is a type of cell death that shows features of both necrosis and apoptosis. It is caspase-independent. It

resembles morphologically necrosis and mechanistically apoptosis.

- **Pyroptosis:** It is a type of programmed cell death accompanied by the release of fever producing cytokine IL-1 and bears some biochemical similarities with apoptosis.
- **Autolysis** (means self-lysis) is destruction of the cell by its own hydrolytic enzymes released from lysosomes. Autolysis is generally reserved for postmortem change. It develops rapidly in some tissues rich in hydrolytic enzymes such as pancreas and gastric mucosa. It occurs little slowly in tissues such as the heart, liver and kidney; and slow in fibrous tissue. Microscopically, the cellular details are lost and they appear as cells with homogeneous and eosinophilic cytoplasm.

Overview of cell injury is presented in Figure 1.26.

PATHOLOGIC CALCIFICATION

Pathological calcification

1. Dystrophic or
2. Metastatic.

Q. Write short note on pathologic calcification.

Abnormal **deposition of calcium salts in tissues other than osteoid or enamel**. It is also associated with deposition of small amounts of iron, magnesium and other minerals.

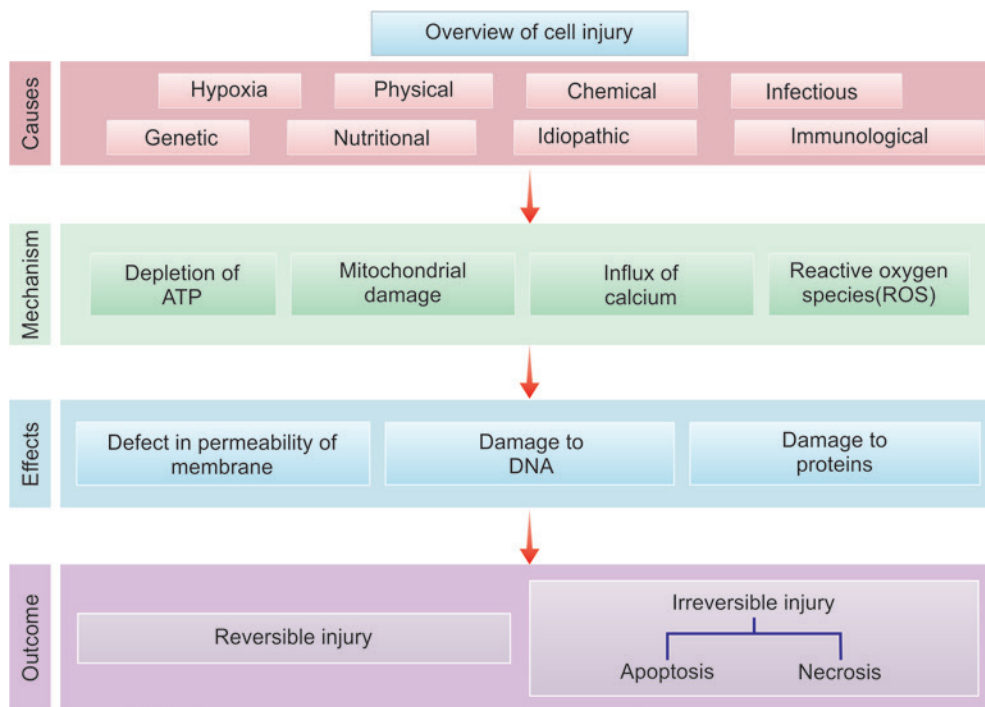


Fig. 1.26: Overview of cell injury

Q. Write short note on psammoma bodies

Psammoma body found in:

1. Papillary carcinoma of thyroid
2. Papillary serous cystadenoma of ovary
3. Papillary serous cystadenocarcinoma of ovary
4. Meningioma
5. Papillary carcinoma of the kidney.

Types of pathologic calcification are: (1) dystrophic and (2) metastatic.

Dystrophic Calcification

Q. Write short note on dystrophic calcification.

Dystrophic calcification:

1. Occurs in dead or degenerating tissues
2. Serum calcium level normal
3. Often causes organ dysfunction.

Deposition of calcium salts in **dying or dead tissues**.

Causes

ABCDE of dystrophic calcification:

- Atherosclerosis
- Psammoma Bodies
- Caseous necrosis
- Damaged heart valves and dead eggs/parasites
- Enzymatic fat necrosis.
- **Necrotic tissue:** Calcification in **caseous, enzymatic fat necrosis**, in **dead eggs** of *Schistosoma*, cysticercosis and hydatid cysts.
- **Degenerating tissue:**
 - **Heart valves:** Occurs in aging or damaged heart valves

– **Atherosclerosis, goiter of thyroid, dense old scar, cysts (e.g. epidermal and pilar cysts of skin).**

- **Monckeberg's medial calcific sclerosis:** Calcification in the media of the muscular arteries (Fig. 1.27A) in old people.
- **Psammoma bodies:** Single necrotic cells on which several layers of mineral get deposited progressively to create lamellated shape called **psammoma bodies** (Fig. 1.27B).

Metastatic Calcification

Q. Write short note on metastatic calcification.

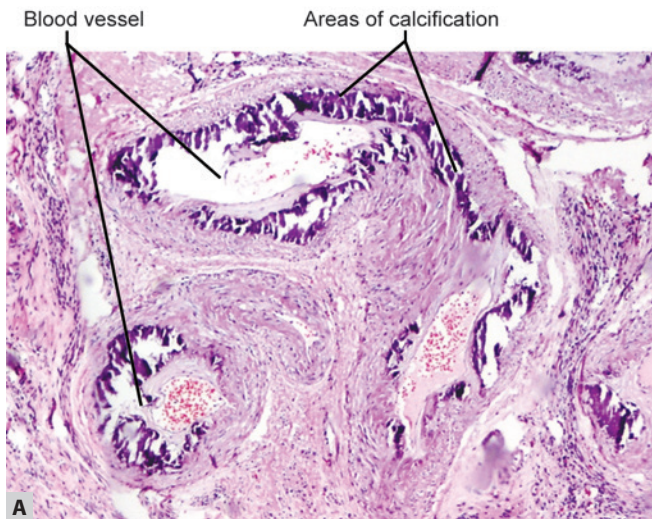
Metastatic calcification

1. Occurs in normal living tissues
2. Associated with raised serum calcium
3. Does not cause clinical dysfunction.

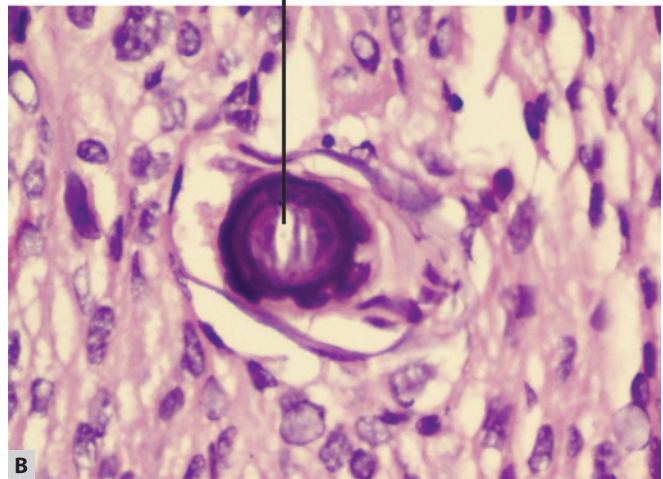
Deposition of calcium salts in apparently **normal tissues**. It is associated with hypercalcemia **secondary to deranged calcium metabolism**.

Causes

- **Increased secretion of parathyroid hormone (PTH)** with subsequent bone resorption-hyperparathyroidism.



Psammoma body consisting of lamellated deposits of calcium



Figs 1.27A and B: (A) Monckeberg's medial calcific sclerosis in which the tunica media of arteries in the myometrium of uterus show calcification; (B) Photomicrograph of meningioma with psammoma body

- **Destruction of bone tissue:** Secondary to primary tumors of bone marrow (e.g. multiple myeloma, leukemia and metastatic tumors to bone).
- **Vitamin D-related disorders:** Vitamin D intoxication.
- **Renal failure:** Causes retention of phosphate, leading to secondary hyperparathyroidism.
- **Others:** Sarcoidosis and milk alkali syndrome.

Sites

Massive deposits of calcium in the kidney is known as nephrocalcinosis and it can lead to kidney damage.

- **Lungs:** Alveolar septa of the lung.
- **Kidney:** Basement membrane of the renal tubules.
- **Blood vessels:** On the internal elastic lamina of systemic arteries and pulmonary veins.
- **Stomach:** Interstitial tissues of the gastric mucosa.

MORPHOLOGY

Common site for metastatic calcification

1. Lungs (commonest site)
2. Kidney
3. Blood vessels (e.g. systemic arteries and pulmonary veins)
4. Stomach.

Gross: Appear as fine, white granules or clumps, feels gritty and sand-like.

Microscopy: Basophilic, amorphous granular (Fig. 1.27), clumped appearance.

HYALINE CHANGE

Q. Write short note on hyaline change.

Hyaline refers to an alteration within cells or in the extra-cellular space, which gives a homogeneous, glassy, pink appearance in routine histological sections.

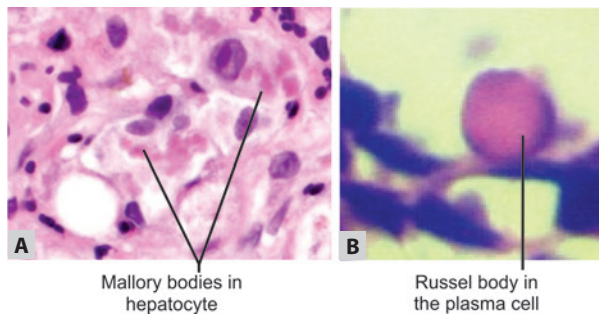
Causes (Table 1.6)

Intracellular Hyaline

- **Mallory body** (Fig. 1.28A) in the liver is alcoholic hyaline composed of cytoskeletal filaments.

TABLE 1.6: Examples of hyaline change

Intracellular hyaline	Extracellular hyaline
1. Mallory bodies	1. Collagenous fibrous tissue in scar
2. Russell bodies (e.g. multiple myeloma)	2. Hyaline change in uterine leiomyoma
3. Crooke's hyaline	3. Hyaline membrane in newborn
4. Zenker's hyaline change	4. Hyaline arteriosclerosis
	5. Hyalinization of glomeruli in chronic glomerulonephritis
	6. Corpora amylacea in prostate, brain, spinal cord in elderly, old infarct of lung



Figs 1.28A and B: A. Mallory bodies; B. Russell bodies

- **Russell bodies** are excessive accumulation of immunoglobulins in the rough endoplasmic reticulum of plasma cells (Fig. 1.28B).
- **Zenker's degeneration:** Hyaline degeneration of rectus abdominis muscle (becomes glassy and hyaline) in typhoid fever.

Mallory hyaline/body observed in:

1. Alcoholic hepatitis
2. Indian childhood cirrhosis (ICC)
3. Primary biliary cirrhosis
4. Wilson disease
5. Hepatocellular carcinoma
6. Focal nodular hyperplasia.

Crooke's hyaline body: Present in basophil cells of pituitary gland in Cushing syndrome.

Extracellular Hyaline

Q. Write short note on Russell bodies.

- Collagenous fibrous tissue in **old scars**.
- Hyaline change in **uterine leiomyoma** (Fig. 1.29).
- In **chronic glomerulonephritis**, the **glomeruli** show hyalinization.

PIGMENTS

Q. Write short note on various pigments.

Pigments are **colored substances**, which are either normal constituents of cells (e.g. melanin), or are abnormal and accumulate in cells. Different types of pigments are listed in Table 1.7.

TABLE 1.7: Different types of pigments

Endogenous pigments	Exogenous pigments
<ul style="list-style-type: none"> • Bilirubin • Melanin • Hemosiderin • Hemoglobin derived pigments 	<ul style="list-style-type: none"> • Carbon (anthracotic) • Tattooing • Arsenic • β-carotene

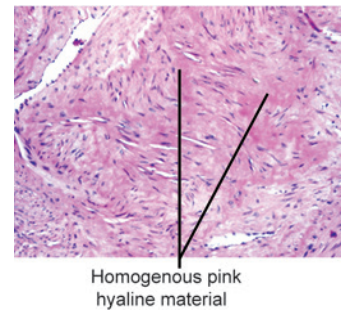


Fig. 1.29: Hyaline change in leiomyoma of uterus

Melanin

Melanin is an endogenous, brown-black, non-hemoglobin-derived pigment. It is produced by the melanocytes and dendritic cells by the oxidation of tyrosine to dihydroxy-phenylalanine by the enzyme tyrosinase. It is stored as cytoplasmic granules in the phagocytic cells namely melanophores. Normally, it is present in the hair, skin, mucosa at some places, choroid of the eye, meninges and adrenal medulla. Various disorders of melanin pigmentation produce generalized and localized hyperpigmentation and hypopigmentation (Table 1.8).

TABLE 1.8: Causes of hyper and hypopigmentation

Generalized hyperpigmentation	Generalized hypopigmentation
<ol style="list-style-type: none"> 1. Addison's disease 2. Chloasma: Hyperpigmentation on the skin of face, nipples, and genitalia during pregnancy. 3. Chronic arsenical poisoning (raindrop pigmentation of the skin) 	<p>Albinism: Generalized hypopigmentation due to genetic deficiency of tyrosinase enzyme</p>
Focal hyperpigmentation	Localized hypopigmentation
<ol style="list-style-type: none"> 1. Café-au-lait spots in neurofibromatosis and Albright's syndrome. 2. Peutz-Jeghers syndrome: Focal peri-oral pigmentation. 3. Melanosis coli: Pigmentation of the mucosa of the colon. 4. Tumors of melanocytes: Benign(nevi) and malignant (melanoma) tumors 5. Lentigo: Premalignant condition 	<ol style="list-style-type: none"> 1. Leukoderma: Autoimmune disorder with localized loss of pigmentation of the skin. 2. Vitiligo: Local hypopigmentation of the skin 3. Acquired focal hypopigmentation: Leprosy, healing of wounds, DLE, radiation dermatitis, pityriasis alba, pityriasis versicolor, idiopathic guttate hypomelanosis, etc.

Alkaptonuria

Q. Write short answer on ochronosis.

Homogentisic acid is a pathological black pigment formed in rare metabolic autosomal recessive disorder termed alkaptonuria. It is characterized by deficiency of an oxidase enzyme needed for breakdown of homogentisic acid. This leads to accumulation of homogentisic acid pigment in the skin, connective tissue, cartilage, capsules of joints, ligaments and tendons. The pigment is melanin-like and the pigmentation is known as **ochronosis**. The homogentisic acid is excreted in the urine (homogentisic aciduria). The urine of patients of alkaptonuria, if allowed to stand for some hours in air, turns black due to oxidation of homogentisic acid.

Hemosiderin

Q. Write short note on hemosiderin and hemosiderosis.

It is a hemoglobin-derived, golden yellow-to-brown, granular or crystalline pigment and is one of the major Russell bodies storage forms of iron.

Causes

Local or systemic excess of iron cause hemosiderin to accumulate within cells.

- **Local excesses:**
 - **Bruise**
 - **Brown induration of lung** in chronic venous congestion of lung (refer Fig. 5.1).
- **Systemic excesses:** **Systemic overload of iron** is known as **hemosiderosis**. The main causes:
 1. **Increased absorption of dietary iron.**
 2. **Excessive destruction of red cells:** For example, hemolytic anemias.
 3. **Repeated blood transfusions.**

MORPHOLOGY

Site of Accumulation

- **Localized:** Found in the macrophages of the involved area.
- **Systemic:** Initially found in **liver, bone marrow, spleen, and lymph nodes**. Later deposited in macrophages of other organs (e.g. skin, pancreas, kidney).

Microscopy: Appears as a coarse, golden, granular pigment within the cytoplasm.

Special stain: **Prussian blue** (Perl's stain) histochemical reaction in which hemosiderin converts colorless potassium ferrocyanide to blue-black ferric ferrocyanide.

Hemosiderin: Golden brown in color. Degradation product of ferritin.

Other Pigments

- **Hemochromatosis:** Severe accumulation of iron is associated with damage to liver, heart and pancreas. The triad of cirrhosis of liver, diabetes mellitus (due to pancreatic damage) and brown pigmentation of skin constitute **bronze diabetes**.
- **Hemozoin:** It is a brown-black pigment containing heme in ferric form. This pigment is seen in chronic malaria and in mismatched blood transfusions.
- **Bilirubin** is the normal major pigment found in bile. It is non-iron containing pigment derived from hemoglobin.
- **Lipofuscin**

Q. Write short note on lipofuscin and brown atrophy of heart.

- **Lipofuscin** is an **insoluble golden-brown endogenous pigment**. It also called as **lipochrome** or **wear and tear pigment**.
- **Composition:** It is composed of mixture of lipids, phospholipids and proteins. **It is accumulated by accretion of peroxidized unsaturated lipids and oxidized cross-linked proteins**. The term lipofuscin is derived from the Latin (*fuscus*, brown), and refers to brown lipid.
- **Significance:** It indicates a product of free radical injury and lipid peroxidation. Lipofuscin does not injure cell or its functions. It is observed in cells undergoing slow, regressive changes and is particularly prominent in the **liver and heart (often called brown atrophy of heart)** of aging patients or patients with severe malnutrition and cancer cachexia.
- **Appearance:** Microscopically, it appears as a yellow-brown, finely granular cytoplasmic pigment, often present in the perinuclear region.

Commonly used histochemistry (special stains) in histopathology are listed in Table 1.9.

Lipochrome/lipofuscin: Wear and tear pigment seen in old age, severe malnutrition, and cancer cachexia. Perinuclear in location. Derived through lipid peroxidation.

Lipofuscin: Important indicator of free radical injury.

Pigmentation of liver may be caused by:

- | | |
|--------------------|------------------|
| 1. Lipofuscin | 4. Bile pigment |
| 2. Malaria pigment | 5. Pseudomelanin |
| 3. Wilson disease | |

CELLULAR AGING

Definition of aging: It is the gradual, insidious and progressive declines in structure and function (involving molecules, cells, tissues, organs and organisms) that begin to unfold after the achievement of sexual maturity.

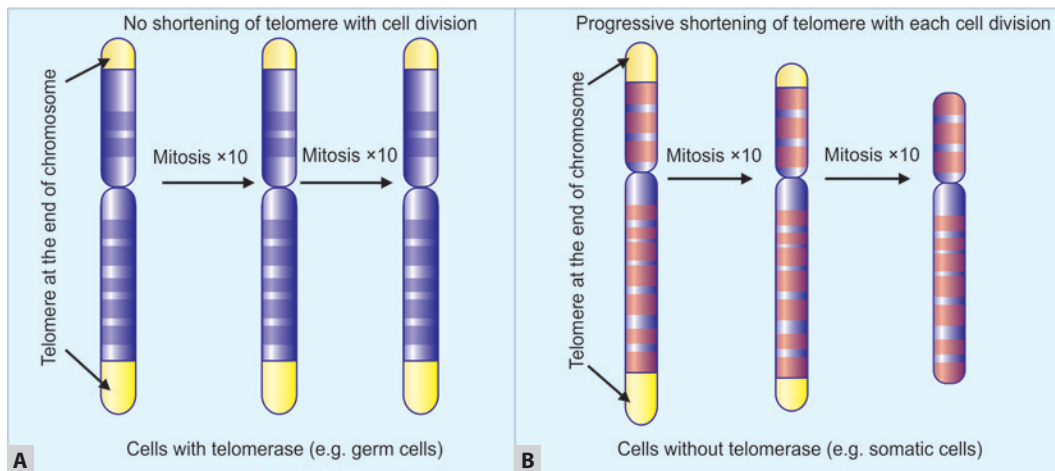
TABLE 1.9: Commonly used special stains in histopathology

Stain	Substance	Interpretation
Amyloid		
<ul style="list-style-type: none">Congo red under polarizing microscope	Amyloid	Green-birefringence
Carbohydrates		
<ul style="list-style-type: none">Periodic acid-Schiff (PAS)	Glycogen, mucin, mucoprotein, glycoprotein, fungi, basement membranes of glomeruli and tubules	Magenta color
<ul style="list-style-type: none">Mucicarmine/Best's carmine	Epithelial mucin	Red color
<ul style="list-style-type: none">Alcian blue	Acid mucin	Blue
Lipids		
<ul style="list-style-type: none">Sudan III	Lipid	Orange
<ul style="list-style-type: none">Oil Red O		Red
<ul style="list-style-type: none">Osmium tetroxide		Brown black
Connective tissue		
<ul style="list-style-type: none">Van Gieson	Extracellular collagen	Red
<ul style="list-style-type: none">Masson's trichrome	Collagen, smooth muscle	Collagen-blue, smooth muscle-red
<ul style="list-style-type: none">Phosphotungstic acid hematoxylin (PTAH)	Cross striation of skeletal muscles, glial filaments, fibrin	Dark blue
<ul style="list-style-type: none">Verhoeff's elastic	Elastic fibers	Black
Microorganisms		
<ul style="list-style-type: none">Gram's stain	Bacteria	Gram+ve = blue Gram-ve = red
<ul style="list-style-type: none">Ziehl-Neelsen's (Acid-fast) stain	Tubercle bacilli and other acid-fast organisms	Red
<ul style="list-style-type: none">Fite-Faraco	Lepra bacilli	Red
<ul style="list-style-type: none">Silver methanamine	Fungi	Black
Pigments and minerals		
<ul style="list-style-type: none">Prussian blue stain (Perl's stain)	Hemosiderin	Blue
<ul style="list-style-type: none">Masson Fontana	Melanin	Black
<ul style="list-style-type: none">Von Kossa	Calcium	Orange red
<ul style="list-style-type: none">Alzarine Red S		Black
<ul style="list-style-type: none">Rubeanic acid	Copper	Greenish-black

Commonest fixative for light microscopic examination: 10% buffered neutral formalin.

Commonest fixative for electron microscopic examination: Glutaraldehyde.

Hematoxyline and eosin (H and E): Routine stain used in histopathology.



Figs 1.30A and B: Role of telomerase in maintaining chromosomal length

Cellular aging begins from conception and continues till death. With aging physiological and structural changes develop in almost all systems. There is progressive loss of functional capacity.

Causes

Aging is multifactorial and is affected by genetic factors and environmental factors.

- **Genetic abnormalities:** It causes progressive decline in cellular function and viability.
- **Environmental factors:** These include diet, social conditions and development of age-related diseases (e.g. atherosclerosis, diabetes and osteoarthritis). They cause progressive accumulation of sublethal injury over the years at cellular and molecular level.
- Cellular aging may lead to death of the cell or decreased capacity of cells to respond to injury and increasing difficulties in maintaining physiological homeostasis.

Mechanism of Cellular Aging

Decreased Cellular Replication

Most normal cells have a limited capacity for replication. After about 60–70 cell divisions, all cells become arrested in a terminally nondividing state, known as **senescence**. **Werner syndrome** is a rare disease characterized by premature aging, damaged DNA and a markedly reduced capacity of cells to divide (shortening of telomere). The following mechanisms may be responsible for progressive senescence of cells and decreased cellular replication in aging.

Telomere Shortening

Telomeres are protective, short repeated sequences of DNA (TTAGGG) present at the end regions of chromosomes.

Telomeres ensure the complete copying of chromosomal ends during the S-phase of the cell cycle. With each cell division in somatic cells, a small section of the telomere is not duplicated and telomeres become progressively shortened (Fig. 1.30). When telomeres are sufficiently shortened, cells stop dividing leading to a terminally nondividing state. Telomeres represent a 'biological clock,' which prevents uncontrolled cell division and cancer. **Telomere shortening** may be one of the mechanisms responsible for decreased cellular replication.

Telomerase

Telomerase is an **enzyme** that **regenerates and maintains telomere length**. Telomerase is absent in most of the somatic cells. Germ cells have high telomerase activity and thus they have extended replicative capacity (Fig. 1.30). In cancers, the telomerase may be reactivated in tumor cells resulting in maintenance of length of telomeres. It may be an essential step in formation of cancer.

Accumulation of Metabolic and Genetic Damage (Fig. 1.31)

Lifespan of the cell is determined by a balance between cumulative metabolic damage and counteracting repair responses.

Metabolic Damage

Reactive oxygen species: One of the toxic products that cause damage to the cells is free radical mainly **reactive oxygen species (ROS)**. ROS may be either produced in excess, or there is reduction of antioxidant defense mechanisms (refer page 13–15).

- **Excessive production of ROS** may be due to environmental influences (ionizing radiation) and mitochondrial dysfunction.

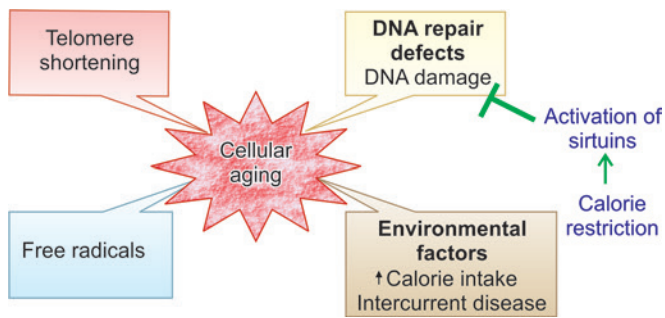


Fig. 1.31: Mechanisms of cellular aging

- **Reduction of antioxidant defense mechanisms** may occur with age (e.g. vitamin E, glutathione peroxidase).

The oxidative damage may be an important cause of senescence in aging. Free radicals may damage DNA, causing breaks and genome instability. Damaged cellular organelles also accumulate as the cells age.

Defective Repair Mechanism

Many protective repair responses counterbalance the metabolic damage in cells. One of them is endogenous DNA repair enzymes, which identify the DNA damage and repairs it. DNA repair mechanisms are defective in diseases such as Werner syndrome and ataxia-telangiectasia.

Thus, aging can be delayed by either by reducing the metabolic damage or by increasing the repair response to that damage.

Factors that Increases Longevity

Caloric Restriction

Calorie restriction prolongs lifespan and this longevity appears to be mediated by a family of proteins known as sirtuin. They have histone deacetylase activity. Red wine can activate sirtuins and thus increase lifespan.

Actions of Sirtuins

- Sirtuins promotes the expression of many genes which increase longevity. The proteins products of these genes increase metabolic activity, reduce apoptosis, stimulate protein folding and inhibit the damaging effects of oxygen-free radicals.
- Sirtuins also increases insulin sensitivity and glucose metabolism.

Growth Factor Signaling

Growth factors, such as insulin-like growth factor trigger the insulin receptor pathway. This results in activation of transcription factors which activate genes that reduce longevity. Mutations in insulin receptor are associated with increased lifespan.

Acute Inflammation

INTRODUCTION

Q. Define inflammation.

Definition: Inflammation is a complex **local response of the living vascularized tissues to injury** and mainly consists of responses of blood vessels and leukocytes.

It brings cells and molecules which are necessary for the defense from the circulation to the sites where they are required. Thus, it try to eliminate the offending injurious agents.

Inflammation is largely confined to the site of infection or damage but can develop some systemic manifestations (e.g. fever in bacterial or viral infections).

Type of inflammation: Inflammation may be divided into **acute or chronic**.

Q. Mention the types of inflammation. List the differences between acute and chronic inflammation.

Differences between acute and chronic inflammation are listed in Table 2.1.

Sometimes, the term subacute inflammation is used to describe the inflammation as between acute and chronic.

Cardinal Signs of Inflammation

Q. Mention the cardinal signs of inflammation and its mechanism.

- The four cardinal signs of inflammation as mentioned by Roman encyclopedist **Aulus Celsus** are listed in Table 2.2.
- A fifth clinical sign, **loss of function** (functio laesa), was later added by Rudolf Virchow.

TABLE 2.1: Differences between acute and chronic inflammation

	<i>Acute inflammation</i>	<i>Chronic inflammation</i>
Onset	Rapid in onset (usually in minutes or hours)	May follow acute inflammation or be slow in onset (days)
Duration	Short duration. Lasts for hours or a few days	Longer duration; may be months
Predominant cells	Neutrophils (also called polymorphonuclear leukocytes)	Lymphocytes, monocytes/macrophages and sometimes plasma cells
Characteristics	Exudation of fluid and plasma proteins (edema) and the emigration of leukocytes	Inflammatory cells associated with the proliferation of blood vessels, tissue destruction and fibroblast proliferation
Injury/damage to tissue and fibrosis	Usually mild and self-limited and can progress to a chronic phase	Usually severe and progressive with fibrosis and scar formation
Signs: Local and systemic	Prominent	Less prominent

Inflammation and the accompanying repair process is a beneficial host response in most instances, but can sometimes be harmful.

TABLE 2.2: Cardinal signs of inflammation (Celsus)

Cardinal sign	Mechanism
Rubor (redness)	Increased blood flow and stasis
Calor (heat)	Increased blood flow
Tumor (edema/ swelling)	Increased vascular permeability causing escape of a protein-rich fluid from blood vessels
Dolor (pain)	Chemical mediators: Prostaglandins and kinins

Russian zoologist Elie Metchnikoff: Phagocytosis.

Sir Thomas Lewis: Triple response.

Julius Cohnheim first described emigration of leukocytes through microvasculature walls inflammation

Causes of (Stimuli for) Acute Inflammation

Q. Mention the various causes of acute inflammation.

- **Infections** (bacterial, viral, fungal, and parasitic) and **microbial toxins**.
- **Tissue necrosis:**
 - **Ischemia:** For example, myocardial infarction
 - **Physical agents**
 - ♦ Mechanical trauma: For example, blunt/penetrating/crush injuries
 - ♦ Thermal injury: For example, burns or frostbite
 - ♦ Radiation
 - ♦ Electric shock
 - ♦ Sudden changes in atmospheric pressure
 - **Chemical injury:** For example, strong acids and alkalies, insecticides, and herbicides
- **Foreign bodies:** For example, sutures, talc
- **Immune reactions:**
 - Hypersensitivity reactions
 - Autoimmune diseases.

SEQUENCE OF EVENTS IN ACUTE INFLAMMATION

Q. Explain the sequential vascular changes/reactions of blood vessels/hemodynamic changes in acute inflammation.

Acute inflammation has two major components namely: (1) **reactions of blood vessels (vascular changes)** and (2) **reactions of leukocytes (cellular events)**.

REACTIONS OF BLOOD VESSELS (VASCULAR CHANGES)

Purpose: To deliver the circulating cells, fluids and plasma proteins from the circulation to sites of infection or tissue injury.

The reactions of blood vessels in acute inflammation (Figs 2.1 and 2.2) consist of: **changes in the vascular flow and caliber** and **increased vascular permeability**.

Changes in Vascular Flow and Caliber

- **Vasodilatation:** It is the earliest feature of acute inflammation; sometimes it follows a transient constriction of arterioles.
 - **Effect:** Result is increased blood flow → local heat and redness.
 - **Chemical mediators involved:** Histamine, prostaglandins, platelet-activating factor, kinins and nitric oxide (NO).
- **Increased permeability of the microvasculature:** It leads to escape of protein-rich fluid from the circulation into the extravascular tissues.
 - **Chemical mediators involved:** Histamine, leukotrienes, platelet-activating factor, and kinins.
- **Slowing of blood flow:** It leads to concentration of RBCs in small vessels and increased viscosity of the blood.
- **Stasis:** It is responsible for localized redness.
- **Leukocyte events:** Described later.

Increased Vascular Permeability (Vascular Leakage)

Q. Write short essay on mechanism of increased vascular permeability (vascular leakage) in inflammation.

Exudation: It is defined as the process of escape of fluid, proteins and circulating blood cells from the vessels into the interstitial tissue or body cavities.

Escape of a protein-rich fluid **causes edema** and is one of the cardinal signs of inflammation. Differences between transudate and exudate are listed in Table 2.3.

Mechanism of Increased Vascular Permeability

Q. Describe the mechanism of increased vascular permeability.

Several mechanisms can cause increased vascular permeability:

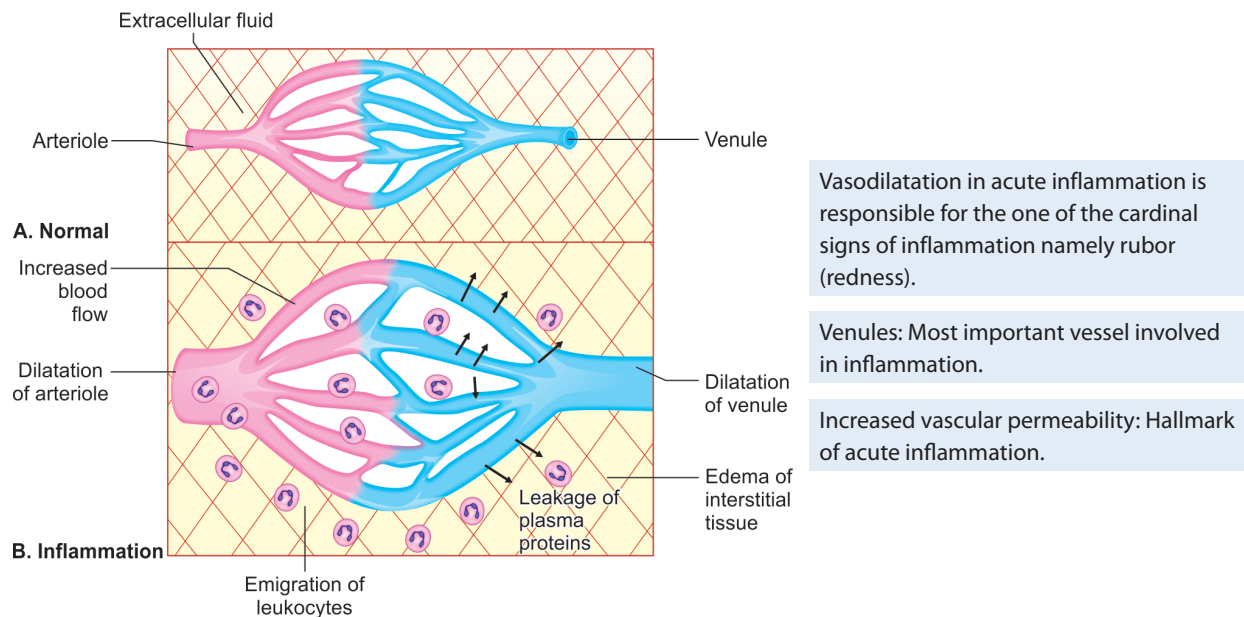
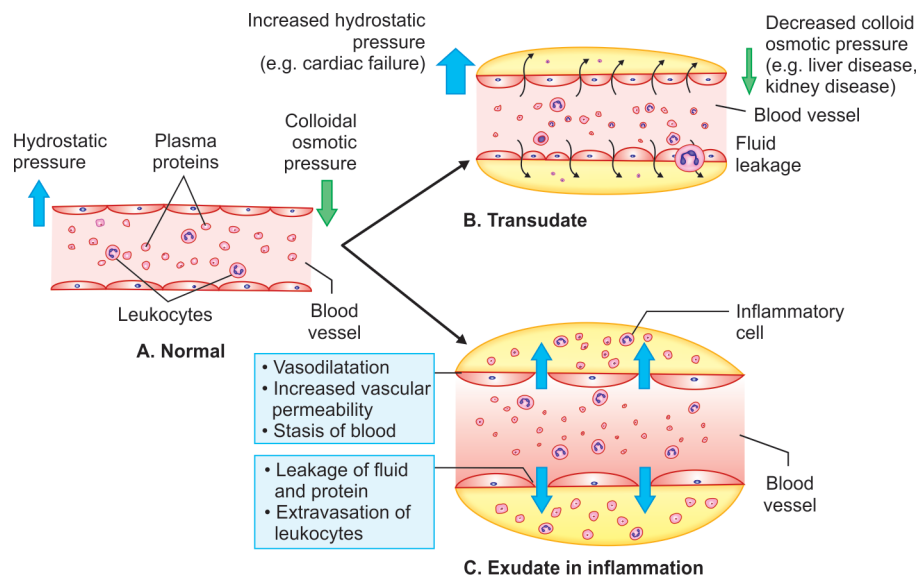


Fig. 2.1: Local features of acute inflammation, compared to normal are vasodilatation, increased blood flow, leakage of plasma fluid and proteins, and emigration of leukocyte



Figs 2.2A to C: (A) Normal fluid exchange between blood and extracellular fluid; (B) Formation of transudate; (C) Formation of exudate in inflammation

Increased vascular permeability causes one of the cardinal signs of inflammation namely tumor (edema).

1. Contraction of endothelial cells:

- Most common mechanism of vascular leakage.
- Occurs immediately after injury and is usually short-lived (15–30 minutes) and hence called as **immediate transient response**.

- **Chemical mediators involved:** Histamine, bradykinin, leukotrienes, the neuropeptide substance P.
- 2. Direct endothelial injury:** For example, burns, or infection by microbes. It is called as immediate sustained response.
- 3. Leukocyte-mediated vascular injury:** The leukocyte (mainly neutrophils) which adheres to the endothelium

Q. Mention the differences between transudate and exudate.

TABLE 2.3: Difference between transudate and exudate

Characteristics	Transudate	Exudate
Cause	Non-inflammatory process	Inflammation process
Mechanism	Ultrafiltrate of plasma, due to increased hydrostatic pressure with normal vascular permeability	Increased vascular permeability
Appearance	Clear, serous	Cloudy/purulent/hemorrhagic/chylous
Color	Straw yellow	Yellow to red
Specific gravity	<1.018	>1.018
Protein	Low, <2 g/dL, mainly albumin	High, >2 g/dL
Clot	Absent	Clots spontaneously because of high fibrinogen
Cell count	Low	High
Type of cells	Few lymphocytes and mesothelial cells	Neutrophils in acute and lymphocytes in chronic inflammation
Bacteria	Absent	Usually present
Lactate dehydrogenase (LDH)	Low	High
Examples	Seen in congestive cardiac failure	Pus
Character of edema	Pitting type	No pitting

during inflammation may themselves injure the endothelial cells.

4. **Increased transcytosis:** Process of transport of fluids and proteins through the channels called vesiculo-vacuolar organelle is increased in number.
5. **Leakage from new blood vessels:** During repair new blood vessels are formed (angiogenesis). These vessels are leaky till the endothelial cells mature.

Increased vascular permeability and chemotaxis: Occurs predominantly in venules (except in lungs, where it occurs in capillaries).

LEUKOCYTIC/CELLULAR EVENTS

Q. Describe leukocyte/cellular events in acute inflammation.

This process **delivers leukocytes** capable of phagocytosis (neutrophils and macrophages) **to the site of injury**. The events can be divided into: **leukocyte recruitment and leukocyte activation**.

Cellular events in acute inflammation:

- Leukocyte recruitment
- Leukocyte activation.

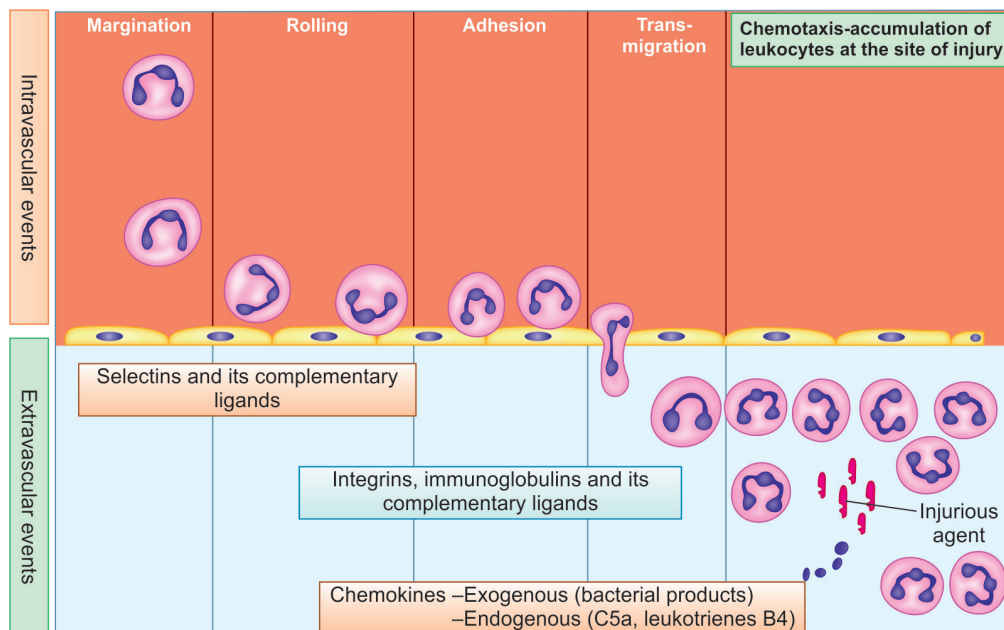
Leukocyte Recruitment/Extravasation

Normally, leukocytes move rapidly in the blood, and during inflammation, they slow down and escape to the site of injury/causative agent in the extravascular space. Leukocyte extravasation is the process of migration of leukocytes from the lumen of the vessel to the site of injury in the extravascular tissues.

Steps in Leukocyte Recruitment/Extravasation (Fig. 2.3)

In the Vascular Lumen

1. **Margination:** When the blood flow slows down (stasis), **leukocytes (mainly neutrophils) move towards the peripheral column** and accumulate along on the endothelial surface of vessels.
2. **Rolling:** Marginated leukocytes attach weakly to the endothelium, detach and bind again with a mild jumping movement. It causes rolling of leukocyte along the endothelial surface.
 - **Molecules involved:** Selectin family of adhesive molecules and its complementary ligands (Table 2.4).
 - **Chemical mediators involved:** Cytokines such as (1) **tumor necrosis factor (TNF)**, (2) **interleukin-1 (IL-1)** and chemokines (chemoattractant cytokines).



Margination is a process in which leukocytes accumulate at the periphery of vessel in early stage of inflammation.

Pus: It is a purulent inflammatory exudate

1. Rich in leukocytes (mostly neutrophils)
2. Debris of dead cells
3. Microbes (in most of the cases).

Acute inflammation:
Main leukocyte involved is neutrophils (polymorphonuclear leukocytes—PMNs).

Fig. 2.3: Various steps of leukocyte migration through blood vessels. The leukocytes first roll, and then firmly adhere to endothelium, followed by transmigration across the endothelium. Leukocytes pierce the basement membrane, and migrate toward chemoattractants from the source of injury. Selectins are involved in rolling; integrins in adhesion; and CD31 (PECAM-1) in transmigration

Q. Describe the role of selectins and integrins in acute inflammation.

TABLE 2.4: Selectins and complimentary selectin ligands involved in rolling

Type of selectin	Distribution	Ligand and their expression
L-selectin (CD62L)	Neutrophils, monocytes	Sialyl-Lewis X/PNAd on GlyCAM-1, CD34, MAdCAM-1
E-selectin (CD62E)	Endothelium activated by cytokines (TNF, IL-1)	Sialyl-Lewis X (e.g. CLA) on glycoproteins; expressed on neutrophils, monocytes, T-cells
P-selectin (CD62P)	Endothelium activated by cytokines (TNF, IL-1), histamine, or thrombin	Sialyl-Lewis X on PSGL-1 and other glycoproteins; expressed on neutrophils, monocytes, T-cells

Abbreviations: GlyCAM-1, glycan-bearing cell adhesion molecule-1; MAdCAM-1, mucosal adhesion cell adhesion molecule-1; TNF, tumor necrosis factor; IL-1, interleukin-1; CLA, Cutaneous lymphocyte antigen-1; PSGL-1, P-selectin glycoprotein ligand-1

Selectins are either not present or expressed at low levels in unactivated endothelial cells.

Process of loose binding and detachment of leukocytes to endothelial cells is termed rolling.

Selectins and its complimentary ligands are responsible for rolling.

During inflammation, the endothelial cells at the site of inflammation gets activated and express high-levels selectins.

3. Adhesion of leukocyte to endothelium: Endothelium gets activated and leukocytes bind more firmly.

- **Molecular involved: Integrins and corresponding ligands** (Table 2.5).

• Chemical mediators involved:

- Endothelial cells are activated by cytokines namely: TNF and IL-1 and increase the expression of two ligands for integrins on leukocyte (Table 2.5).
- Chemokines are chemoattractant cytokines cause leukocyte activation and **conversion of low-affinity integrins on leukocyte to high-affinity state** resulting in firm adhesion of the leukocytes to the endothelium.

Integrins are responsible for firm adhesion of leukocytes with endothelial cells.

TABLE 2.5: Integrins and complimentary ligands involved in endothelial-leukocyte adhesion

Type of integrins	Distribution	Complimentary ligands expressed on endothelium
$\beta 1$ integrin VLA-4 (CD49aCD29)	Monocytes, T-cells	VCAM-1 (CD106)
$\beta 2$ integrins LFA-1 (CD11aCD18)	Neutrophils, monocytes, T-cells	ICAM-1 (CD54), ICAM-2 (CD102)
$\beta 2$ integrins MAC-1 (CD11bCD18)	Monocytes, dendritic cells	ICAM-1 (CD54), ICAM-2 (CD102)

Abbreviations: ICAM, Inter cellular adhesion molecule; VCAM, Vascular cell adhesion molecule.

Across the Vessel Wall and the Endothelium

Q. Write short note on leukocyte transmigration.

1. **Transmigration or diapedesis:** Leukocytes migrate through the vessel wall by squeezing through the intercellular junctions between the endothelial cells.
 - **Molecules involved:** These include a member of the immunoglobulin superfamily called CD31 or PECAM-1 (platelet endothelial cell adhesion molecule).
2. **Migration across the basement membrane:** Leukocytes penetrate the basement membrane of the vessel by secreting collagenases.

Outside the Vessel Wall

Q. Define and write short note on chemotaxis.

1. **Chemotaxis.**

Definition: Chemotaxis is defined as process of migration of leukocytes toward the inflammatory stimulus in the direction of the gradient of locally produced chemoattractants.

Chemoattractants:

- **Exogenous:** Bacterial products (e.g. N-formylmethionine terminal amino acid).
- **Endogenous:**
 - Cytokines, mainly chemokine family (e.g. IL-8)
 - Complement components: C5a, C3a
 - Arachidonic acid metabolites of lipoxigenase pathway: **Leukotriene B₄ (LTB₄)**.

Chemotaxis is the **unidirectional movement of leukocytes** towards injurious agent.

2. **Accumulation of leukocytes at the sites of infection and injury:** Achieved by binding of leukocytes to the extracellular matrix proteins through integrins and CD44.
 - **Type of leukocytes infiltrates:**
 - **Neutrophils:** Predominantly during the first 6–24 hours.
 - **Monocytes:** Neutrophils are replaced by monocytes in 24–48 hours.

Acute inflammation: Neutrophils predominate in early stage and are replaced by monocytes after 24 hours.

Pseudomonas infection: Neutrophils predominate over 2 to 4 days

Clinical Importance of Leukocyte Adhesion Molecules

- Three main types of leukocyte adhesion deficiency (LAD) have been identified.
- All are transmitted as autosomal recessive disease.
- Characterized by the inability of neutrophils to exit the circulation to sites of infection, leading to **leukocytosis** and **increased susceptibility to infection**.

Genetic deficiencies of leukocyte adhesion molecules cause recurrent bacterial infections.

Leukocyte adhesion deficiency type 1 (LAD1)

- Integrin defects
- Recurrent infections
- Persistent leukocytosis
- Delayed separation of umbilical stump.

Leukocyte adhesion deficiency type 2 (LAD2):

- Selectin defects
- Recurrent infections
- Bombay blood group
- Mental retardation

Leukocyte adhesion deficiency type 3 (LAD3):

- Caused by mutations in the gene *FERMT3*.
- Impaired integrin activation
- Increased susceptibility to infection
- Leukocytosis, and petechial hemorrhage.

Leukocyte Activation

Activation of leukocytes: Recognition of microbes or dead cells by the leukocyte receptors initiates several responses in leukocytes together known as **leukocyte activation**. The most important functional responses of leukocyte activation is phagocytosis and intracellular killing.

Develops in two sequential events:

Recognition of microbes, necrotic cells and foreign substances: Leukocytes recognize microbes, necrotic cells and foreign substances by cell surface receptors known as “**pattern recognition receptors**”. The most important of these receptors are:

- **Toll-like receptors (TLRs):** They can recognize extracellular and ingested microbes, like bacterial lipopolysaccharide (LPS, or endotoxin).
- **Inflammasome:** It is a multiprotein complex and can recognize products of dead cells (e.g. uric acid, microbial products). Triggering of inflammasome causes activation of IL-1. This in turn recruits leukocytes, which phagocytose and destroy dead cells. IL-1 plays a role in atherosclerosis and obesity-associated type 2 diabetes mellitus. These findings suggest that IL-1 antagonists may be useful in treating such diseases.

Inflammasome: Multiprotein complex and can recognize products of dead cells (e.g. uric acid, microbial products).

Phagocytosis and Clearance of the Offending Agent

Q. Write short note on phagocytosis and its sequence of events.

Many leukocytes **recognize, internalize, and digest** foreign material, microorganisms, or cellular debris by a process termed phagocytosis. It consists of three steps (Figs 2.4A to C):

- Recognition and attachment
- Engulfment
- Killing or degradation of the ingested material.

Phagocytosis: Process by which recognition, internalization and digestion of foreign material, microorganisms, or cellular debris occurs.

Recognition and Attachment

- Receptors on the surface of phagocytic cells recognize components of microbes and necrotic cells. Leukocytes

express several receptors that recognize external stimuli. These include (1) receptors for microbial products (e.g. Toll-like receptors-TLRs), (2) G protein-coupled receptors (recognize N-formyl methionine residues), (3) receptors for cytokines (for $\text{INF-}\gamma$) and (4) receptors for opsonins (described below).

Q. Write short note on opsonins and their role in inflammation.

- **Receptors for opsonins (phagocytic receptor):** The phagocytosis is enhanced when leukocyte receptors recognize microbes coated by specific host proteins known as **opsonins**. The major opsonins are IgG antibodies, the C3b breakdown product of complement, and certain plasma lectins called collectins (Table 2.6).

Opsonization: Process of coating of a particle (e.g. microbe), by opsonins to increase its **phagocytosis**.

TABLE 2.6: Different opsonins and their corresponding receptors on leukocyte

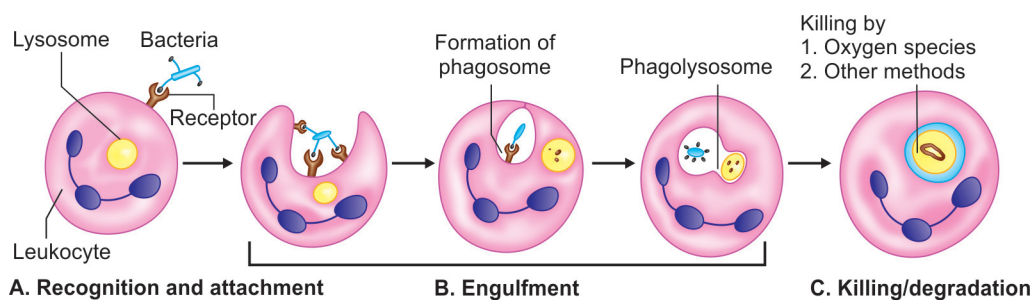
Opsonin	Receptor on leukocyte
IgG antibodies	Fc receptor (FcγRI):
Complement components C3	Type 1 and 3 complement receptor (CR1 and CR3)
Collectins	C1q

Opsonins include:

- Antibodies
- Complement fragment C3b
- Acute phase proteins (e.g. CRP)
- Collectins
- Mannose-binding lectins

Clinical significance of opsonins:

After exposure to antigen, B cells get activated and mature into plasma cells, which produce immunoglobulins (IgG).



Figs 2.4A to C: Different steps in phagocytosis. (A) Recognition and attachment which involves binding to receptors on the leukocyte membrane to injurious agent (e.g. bacteria); (B) Engulfment, formation of phagosome and fusion of lysosomes with phagocytic vacuoles to form phagolysosome; (C) Killing/degradation of ingested particles within the phagolysosomes by lysosomal enzymes and by reactive oxygen and nitrogen species

Nitroblue tetrazolium test: Used for phagocytosis. Most widely used test for chronic granulomatous disease.

Neutrophils in acute inflammation: Cleared by apoptosis

Pinocytosis (cell drinking) and receptor mediated endocytosis: Requires clathrin coated pits.

Bruton disease: Defect in maturation of the B-cells leading to absence of immunoglobulin production. Hence, there is **defective opsonization**.

Engulfment

Next step in phagocytosis is engulfment and formation of a phagocytic vacuole. Phagocytosis is dependent on polymerization of actin filaments.

- **Phagosome:** Extensions of the **cytoplasm of leukocyte form pseudopods surrounding the particle to be ingested** and forms a vesicle or vacuole called a phagosome.
- **Phagolysosome:** The membrane of **phagosome fuses** with membrane of **lysosome to form a phagolysosome**. Lysosomal **granules are discharged** into this phagolysosome.

Phagocytosis (cell eating): Requires polymerization of actin filaments in the leukocytes.

Clinical significance of defects in phagolysosome function:

Chédiak-Higashi syndrome: Autosomal recessive condition characterized by:

- **Increased susceptibility to infections:** Due to **defective fusion of phagosomes and lysosomes in phagocytes**.
- Leukocyte abnormalities include:
 - Neutropenia (decreased numbers of neutrophils)
 - Defective degranulation and delayed microbial killing
 - Peripheral blood smear: **Leukocytes contain giant granules**, due to aberrant phagolysosome fusion.
- Gene associated with this syndrome encodes a **large cytosolic protein** called **LYST**, which **regulates lysosomal trafficking**.
- Albinism: Due to abnormalities in melanocytes.
- Nerve defects.
- Bleeding disorders due to defect in platelets.

Chediak-Higashi syndrome: No formation of phagolysosome. Leukocytes have giant granules due to aberrant fusion of organelle.

Killing and Degradation

Killing and degradation of ingested microbial agents/particles occurs within neutrophils and macrophages. Most important microbicidal agents are: (1) reactive oxygen species (2) reactive nitrogen species-derived from nitric oxide (NO), and (3) lysosomal enzymes.

Reactive oxygen species (ROS):

Q. Write short note on free radicals and acute inflammation.

Oxygen dependent killing in leukocytes is done through generation of ROS by NADPH oxidase present in the leukocytes.

Types of ROS are:

- Superoxide anion (O_2^- , one electron)—**weak**
- Hydrogen peroxide (H_2O_2 , two electrons)—**weak**
- Hydroxyl ions ($\cdot OH$), three electrons—**highly reactive**.

Mechanism of production (refer pages 13 to 15): In the phagocytic vacuole of leukocyte, rapid activation of NADPH oxidase (also called **phagocyte oxidase**), oxidizes NADPH (reduced nicotinamide-adenine dinucleotide phosphate) to NADP. During the process oxygen is reduced to superoxide anion (O_2^-).

- O_2^- is converted into hydrogen peroxide (H_2O_2) by spontaneous dismutation



- Amount of H_2O_2 is insufficient to kill most of the microbes by itself but the enzyme **myeloperoxidase** (MPO) present in the azurophilic granules of neutrophils can convert it into a powerful ROS. MPO in the presence of a halide such as Cl^- , converts H_2O_2 to hypochlorous radical ($HOCl^\cdot$), which is a potent oxidant and antimicrobial agent. Hypochlorite ($HOCl^\cdot$) destroys microbes either by **halogenation** or by proteins and lipid peroxidation.
- H_2O_2 is also converted to hydroxyl radical ($\cdot OH$) which is also powerful destructive agent.

Oxygen dependent MPO system is the **most powerful microcidal mechanism**.

Hypochlorite ($HOCl^\cdot$)

1. Active component of bleach
2. It is an end product of oxygen dependent MPO system.

Reactive nitrogen species:

NO, which is generated from arginine by the action of nitric oxide synthase (NOS), can kill microbes similar to ROS.

Q. Write short note nitric oxide in inflammation.

$\cdot NO$ reacts with superoxide (O_2^-) and produces highly reactive free radical peroxynitrite ($ONOO^\cdot$).

Phagocytosis by leukocytes can destroy or remove the microbes and dead cells.

Lysosomal enzymes:

Acid hydrolases of lysosomes degrade the dead microorganisms. Elastase can kill bacteria.

- **Constituents of leukocyte granules:** The microbicidal substances within leukocyte cytoplasmic granules include:
 - Bactericidal permeability—increasing protein
 - Lysozyme and lactoferrin

- **Major basic protein (MBP) present in eosinophils is cytotoxic to many parasites**
- Defensins are toxic to microbes
- Cathelicidins: These are antimicrobial proteins in the neutrophils and other cells. They are very effective against *Mycobacterium tuberculosis*.

Neutrophil secretes cathepsin G.

Examples of leukocyte-induced injury:

- Acute: For example, acute respiratory distress syndrome, glomerulonephritis
- Chronic: For example, rheumatoid arthritis, atherosclerosis.

Neutrophil Extracellular Traps

In response to infectious agents and inflammatory mediators neutrophils may produce an extracellular fibrillary networks known as “traps”. Neutrophil extracellular traps (NETs) contain nuclear chromatin (histones and DNA) with granule proteins (e.g. antimicrobial peptides and enzymes). These traps prevent the spread of microbes by trapping them in the fibrils.

Clinical Significance of Inherited Defects in Microbicidal Activity

1. **Chronic granulomatous disease (CGD):** Group of congenital (inherited) disorders characterized by defects in bacterial killing.
 - **Decreased oxidative burst:** Defects in the genes encoding components of **phagocyte oxidase (NADPH oxidase)** which generates superoxide anion (O_2^-). Variants of phagocyte oxidase are:
 - X-linked defect: Defect in the gene coding membrane component of NADPH/phagocyte oxidase.
 - Autosomal recessive: Defect in the gene coding cytoplasmic component of NADPH/phagocytic oxidase.
 - Susceptible to recurrent bacterial infection.
 - Disease named granulomatous because the initial neutrophil defense is inadequate and there is **chronic inflammatory reaction rich macrophage** that tries to control the infection. These collections of activated macrophages try to wall off the microbes, forming aggregates called granulomas.
 - **Diagnosis of CGD:**
 - **Nitroblue-tetrazolium (NBT) test:** This test depends on the direct reduction of NBT by superoxide anion (O_2^-) to form an insoluble formazan. It is positive in normal individuals (with NADPH oxidase), but negative in CGD.

- **Dihydrorhodamine (DHR) test:** In this test, whole blood is stained with DHR, incubated and stimulated to produce superoxide anion (O_2^-). This free radical reduces DHR to rhodamine in cells with normal NADPH oxidase.
- **Cytochrome C reductase assay:** This is an advanced test that quantifies the amount of superoxide anion (O_2^-) that can be produced by patient's phagocytes.

2. **MPO deficiency:** Decreased microbial killing because of defective MPO— H_2O_2 system.

Genetic or acquired defects in leukocyte function: Recurrent infections.

In genetic deficiency of MPO, the increased susceptibility to infection is due to: Inability to produce hydroxyl-halide radicals.

Chronic granulomatous disease (CGD) is characterized by absence of NADPH oxidase and respiratory burst. (Repeated infections by catalase +ve organisms, bacterial infections by *Staphylococcus aureus* and fungal due to *Candida*).

Acquired Defects of Leukocyte Functions

- **Decreased production of leukocytes:** For example, bone marrow suppression (tumors, radiation, and chemotherapy).
- **Defect in leukocyte adhesion and chemotaxis:** For example, diabetes, malignancy, sepsis, chronic dialysis.
- **Defects in phagocytosis and microbicidal activity:** For example, leukemia, anemia, sepsis, diabetes, malnutrition.

CHEMICAL MEDIATORS OF INFLAMMATION

Q. List chemical mediators of inflammation. Role of chemical mediators in inflammation. Name the cell derived mediators of inflammation. Name the plasma-derived mediators of inflammation.

Numerous chemical mediators are responsible for inflammatory reactions.

General Features of Chemical Mediators

- **Source of mediators:** Mediators are derived either from cells or from plasma proteins (Table 2.7).
 - **Cell-derived mediators:**
 - ◆ Present either as preformed molecules (e.g. histamine in mast cell granules) or are synthesized de novo (e.g. prostaglandins, cytokines) in response to a stimulus.

Q. Name the cell-derived mediators of inflammation.

TABLE 2.7: Main chemical mediators of acute inflammation

Cell-derived	Plasma protein-derived
<ul style="list-style-type: none"> • Vasoactive amines <ul style="list-style-type: none"> – Histamine – Serotonin 	<ul style="list-style-type: none"> • Complement components <ul style="list-style-type: none"> – C3a – C5a – C3b – C5b-9 (MAC)
<ul style="list-style-type: none"> • Arachidonic acid (AA) metabolites <ul style="list-style-type: none"> – Prostaglandins – Leukotrienes 	<ul style="list-style-type: none"> • Kinins <ul style="list-style-type: none"> – Bradykinin – Kallikrein
<ul style="list-style-type: none"> • Platelet-activating factor (PAF) 	<ul style="list-style-type: none"> • Coagulation/fibrinolytic system
<ul style="list-style-type: none"> • Reactive oxygen species (ROS) 	
<ul style="list-style-type: none"> • Nitric oxide (NO) 	
<ul style="list-style-type: none"> • Cytokines (TNF, IL-1) and Chemokines 	

Abbreviation: IL-1, interleukin-1; TNF, tumor necrosis factor; MAC, membrane attack complex.

- ♦ Produced usually by platelets, neutrophils, monocytes/macrophages, and mast cells.
- **Plasma-derived mediators:** Produced mainly in the liver and present in the circulation as **inactive precursors, which require activation** (e.g. complement proteins, kinins).
- **Tightly regulated actions.**
- **Inter-related:** One mediator can stimulate the release of other mediators. The secondary mediators may have the similar, different or even opposite actions.
- Most act by **binding to specific receptor** on target cells.
- **Diverse targets:** Target cell type varies depending on the type of mediator. They can act on one or few or many diverse targets, or may have different effects on different types of cells.
- **Short-lived:** Most of these mediators have a short-lifespan.

Chemical mediators: Most of them have short-lifespan.

Main mediators involved in the inflammatory reaction are listed in Table 2.7.

Vasoactive amines namely histamine and serotonin cause **vasodilatation and increased vascular permeability**.

Chemical mediators:

1. Cell-derived or
2. Plasma protein-derived.

Cell-Derived Mediators

Q. Write short note on cell-derived mediators of inflammation.

Vasoactive Amines: Histamine and Serotonin

Histamine and serotonin are the first mediators to be released during inflammation, which are stored as preformed molecules in cells.

1. **Histamine:** It is a preformed vasoactive mediator. Responsible for immediate transient response.

Source: Mast cells (richest source), blood basophils and platelets.

• **Stimuli:**

- Physical injury (e.g. trauma, cold, heat)
- Immune reactions in which antibodies bind to mast cells (e.g. allergic reactions)
- Other chemical mediators: C3a and C5a, leukocyte-derived histamine-releasing proteins, neuropeptides (e.g. substance P), cytokines (IL-1, IL-8).

Actions: (1) Dilation of arterioles and (2) increase of the vascular permeability.

2. **Serotonin (5-hydroxytryptamine):** It is a preformed vasoactive mediator.

- **Source:** Platelets, some neurons and enterochromaffin cells in the gastrointestinal tract.
- **Stimulus:** Platelet aggregation and antigen-antibody complexes.
- **Actions:** Similar to those of histamine.

Histamine and serotonin are preformed vasoactive cell-derived mediators.

Arachidonic Acid Metabolites

(Prostaglandins, Leukotrienes, and Lipoxins)

Q. Write short note on role of arachidonic acid metabolites in inflammation.

Arachidonic Acid (AA)

Arachidonic acid: Can be enzymatically converted into prostaglandins and leukotrienes (both together called as eicosanoids).

- **Source:** Derived from cell membrane phospholipids mainly by the enzyme phospholipase A₂.
- **Stimuli:** Mechanical, chemical, and physical stimuli or other mediators (e.g. C5a).

- **AA metabolism:** Occurs along two major enzymatic pathways (Fig. 2.5). These are **cyclooxygenase pathway** (produce prostaglandins) and **lipoxygenase pathway** (produces leukotrienes and lipoxins).

A. Products of cyclooxygenase pathway:

- **Products:** Most important in inflammation are PGE_2 , PGD_2 , PGI_2 (prostacyclin), and TXA_2 (thromboxane A_2).

Q. Write short note on role of prostaglandin in acute inflammation.

- **Mechanism:** They are produced from AA by the actions of two **cyclooxygenases**, COX-1 and COX-2.
- **Local effects:**
 - TxA_2 : **Vasoconstriction** and promotes **platelet aggregation**
 - **Prostacyclin (PGI_2)**: **Vasodilator** and **inhibits platelet aggregation**

- PGD_2 and PGE_2 : **Vasodilation and increased permeability**. PGD_2 is also a chemoattractant for neutrophils.

• Systemic effects:

- Prostaglandins are responsible for **pain and fever** in inflammation.
- PGE_2 causes cytokine-induced **fever** during infections.

B. Products of lipoxygenase pathway: (1) Leukotrienes and (2) lipoxins.

1. Leukotrienes: Products and their actions:

- **5-hydroxyeicosatetraenoic acid (5-HETE):** Chemotactic for neutrophils, and is the precursor of the leukotrienes.
- LTB_4
 - **Chemotactic agent**

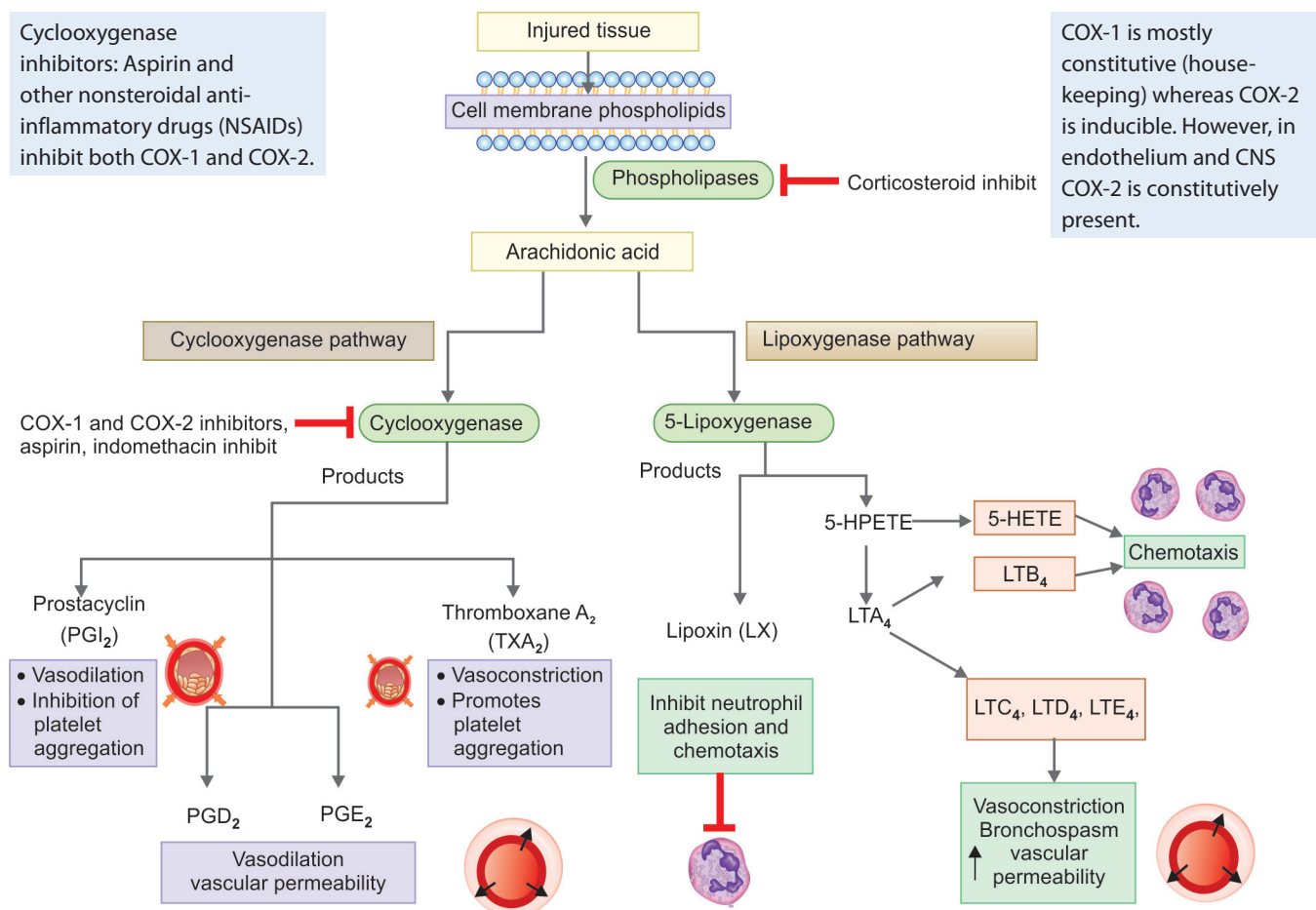


Fig. 2.5: Arachidonic acid metabolites involved in inflammation. The cyclooxygenase pathway generates prostaglandins (PGIs) and thromboxane (TXA_2). The lipoxygenase pathway forms lipoxins (LXs) and leukotrienes (LTEs).

Abbreviation: COX, cyclooxygenase; HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid.

- Neutrophil activation causing adhesion to endothelium, generation of ROS, and release of lysosomal enzymes.

- **Leukotrienes C₄, D₄, and E₄** (LTC₄, LTD₄, LTE₄)

- Vasoconstriction
- Bronchospasm (in asthma)
- **Increased vascular permeability.**

2. Lipoxins (LXs):

- **Actions: Inhibit inflammation**

- **Inhibit neutrophil chemotaxis** and recruitment.
- **Inhibit leukocyte adhesion** to endothelium.

Main actions of arachidonic acid metabolites (eicosanoids) involved in inflammation are presented in Table 2.8.

Many anti-inflammatory drugs act by inhibiting the synthesis of eicosanoids.

PGI₂: Inhibition of platelet aggregation (I = inhibition)

TXA₂: Platelet aggregation (A = aggregation).

PGF_{2α}: Vasoconstrictor

PGD₂ and PGE₂: Vasoconstrictors.

TABLE 2.8: Main actions of arachidonic acid metabolites (eicosanoids) in inflammation

Action	Arachidonic acid metabolites (Eicosanoid)
Vasodilation	PGI ₂ (prostacyclin I ₂), PGE ₂ , PGD ₂
Increased vascular permeability	Leukotrienes C ₄ , D ₄ , E ₄
Chemotaxis, leukocyte adhesion	Leukotriene B ₄ , HETE (hydroxyeicosatetraenoic acid)

Arachidonic acid products: Can mediate almost every step of inflammation.

Broad-spectrum inhibitors: Corticosteroids reduce the transcription of genes encoding COX-2, phospholipase pro-inflammatory cytokines (such as IL-1 and TNF), and iNOS.

Lipoxygenase inhibitors: Drugs which inhibit leukotriene production (e.g. Zileuton) or block leukotriene receptors (e.g. Montelukast) are used in the treatment of asthma.

Q. Write short note on platelet-activating factor.

Platelet-activating Factor (PAF)

Action: Multiple inflammatory effects:

- **Vascular reactions:** Vasodilation and increased vascular permeability.
- **Cellular reactions:** Increased leukocyte adhesion to endothelium, chemotaxis.

- **Others:** Increases the synthesis of other mediators, mainly eicosanoids.

Reactive Oxygen Species

ROS: Cause killing of microbes and tissue damage.

Q. Write short note on free radicals and acute inflammation.

Reactive oxygen species (ROS) are chemically reactive oxygen-derived free radical. Normally, they are rapidly inactivated. But increased production can cause cell injury.

Cell of origin: Leukocytes (neutrophils and macrophages).

Mechanism of production: Leukocytes during phagocytosis (after exposure to microbes, chemokines, and immune complexes) generate oxygen-derived free radicals (refer Figs 1.10 and 1.11).

Types: Superoxide anion (O₂^{•−}), hydrogen peroxide (H₂O₂), and hydroxyl radical (•OH).

O₂^{•−} can combine with NO to form reactive nitrogen species (peroxynitrite ONOO[−]).

Actions:

- **Physiologic function:** ROS in leukocytes destroys phagocytosed microbes and necrotic cells.
- **Pathological actions:**
 - Endothelial cell damage, which causes increased vascular permeability.
 - Injury to other cells: For example, tumor cells, parenchymal cells and red blood cells.
 - Inactivation of antiproteases, such as α₁-antitrypsin, e.g. destruction of elastic tissues in emphysema of lung.

Nitric Oxide

Q. Write short note on nitric oxide in inflammation.

Nitric oxide (NO) is a soluble, free radical gas which causes vasodilation (was known as endothelium-derived relaxing factor).

Source: Many cells such as endothelial cells, macrophages and neurons in the brain.

Synthesis: Synthesized from L-arginine, molecular oxygen, and NADPH by the enzyme nitric oxide synthase (NOS).

Types: 3 isoforms of NOS: Type I neuronal (nNOS), type II inducible (iNOS) and type III endothelial (eNOS).

Action (Fig. 2.6): It acts in a paracrine manner on target cells.

- **Vasodilation** by relaxing vascular smooth muscle cells.
- **Controls inflammatory responses** by inhibiting leukocyte recruitment and adhesion.
- **Reduced platelet adhesion, aggregation and degranulation**
- **Microbicidal activity.**

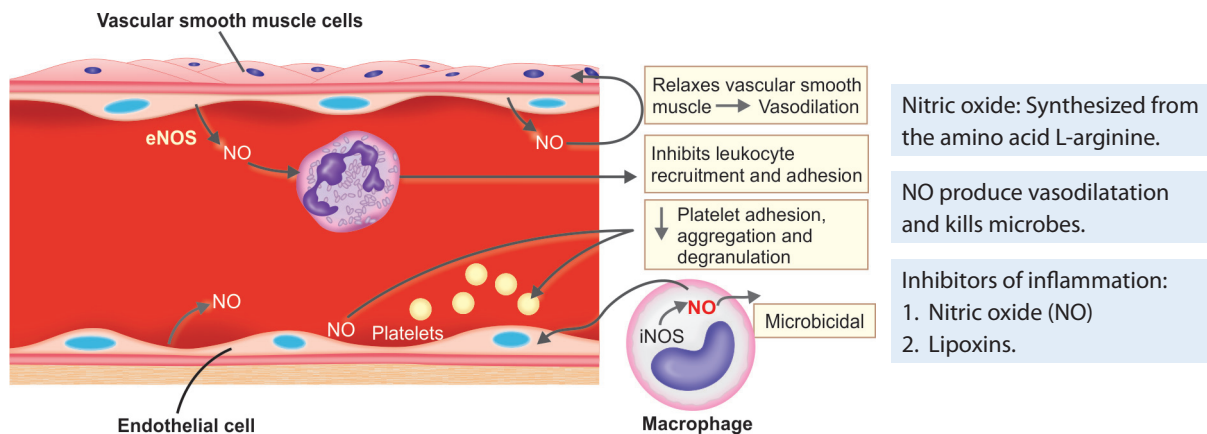


Fig. 2.6: Role of nitric oxide (NO) in blood vessels and macrophages. NO is produced by NO synthase enzymes. It causes vasodilation, and NO-derived free radicals are microbicidal

Cytokines and Chemokines

Cytokines are soluble proteins that mediate immune responses and inflammation.

Q. Write short note on cytokines.

These are polypeptides which function as mediators in **immune responses** and in **inflammation (acute and chronic)**.

Source: Cytokines are secreted by many types of cell (activated lymphocytes and macrophages, endothelial, epithelial, and connective tissue cells).

Cytokines exert their effect by binding to specific receptors on target cells.

Cytokines play multiple roles in inflammation. Causes endothelial activation and fever.

Tumor Necrosis Factor and Interleukin-1

These are the two major cytokines involved in inflammation.

Source: Activated macrophages.

Stimuli: Endotoxin and other microbial products, immune complexes, physical injury, and many inflammatory stimuli.

Actions in inflammation (Fig. 2.7):

- **Local effects:**
 - **Endothelium:** Endothelial activation and increased expression of **endothelial adhesion molecules**.
 - **Leukocytes:** TNF increases the responses of neutrophils to other stimuli (e.g. bacterial endotoxin).
 - **During repair:** Proliferation of fibroblasts and increased synthesis of collagen.
- **Systemic effects:**
 - Fever

- Leukocytosis
- Systemic acute-phase reactions
- Suppresses appetite: TNF contributes to cachexia seen in some chronic infections.

Chemokines

Chemotactic cytokines or chemokines are small proteins, which selectively attracts various leukocytes to the site of inflammation.

Classification: Chemokines are classified four major groups namely: (1) C-X-C chemokines, (2) C-C chemokines, (3) C chemokines and (4) CX3C chemokines.

Action: Chemotaxis of monocytes, eosinophils, basophils, and lymphocytes except neutrophils. They activate leukocyte and promote their recruitment to the sites of inflammation.

Some chemokine receptors (CXCR-4, CCR-5) act as coreceptors involved in binding and entry of the human immunodeficiency virus into lymphocytes.

IL-10 and TGF- β : Possess anti-inflammatory action. TGF- β is the most important fibrogenic agent.

Other Cytokines in Acute Inflammation

Main cytokines involved in acute inflammation are: TNF, IL-1 and IL-6.

Chemokines are chemotactic and also cause leukocyte activation.

- IL-6 produced by macrophages and other cells is involved in local and systemic reactions.
- IL-17 produced by T lymphocytes promotes neutrophil recruitment.

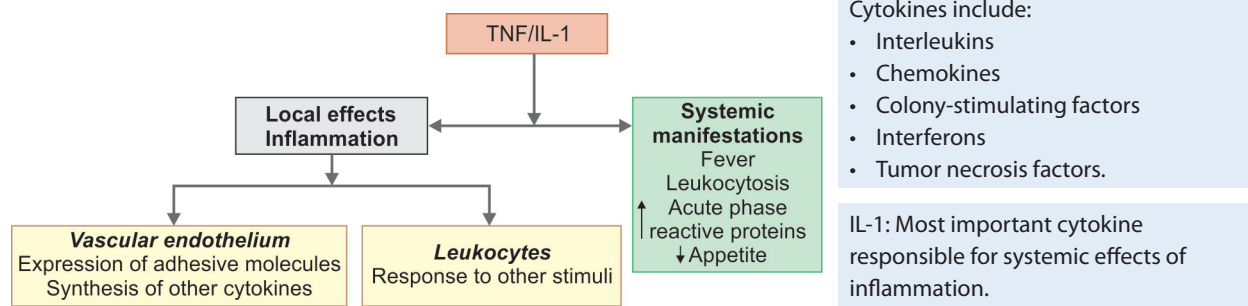


Fig. 2.7: Important local and systemic effects of tumor necrosis factor (TNF) and interleukin-1 (IL-1)

Lysosomal Constituents of Leukocytes

Neutrophils

Types of granules:

1. **Smaller specific (or secondary) granules:** They contain lysozyme, collagenase, gelatinase, lactoferrin, plasminogen activator, histaminase, and alkaline phosphatase.
2. **Larger azurophil (or primary) granules:** They contain myeloperoxidase, bactericidal factors (lysozyme, defensins), acid hydrolases, and a variety of neutral proteases (elastase, cathepsin G, nonspecific collagenases, proteinase 3).

Lysosomal enzymes:

- Microbial killing
- Tissue injury

Monocytes and Macrophages

They also contain acid hydrolases, collagenase, elastase, phospholipase, and plasminogen activator. These are active mainly in chronic inflammation.

Neuropeptides

- These are small peptides, such as substance P and neurokinin A.
- **Source:** Secreted by sensory nerves and various leukocytes.
- **Action:** Vasodilation and increased vascular permeability.

Plasma-Derived Mediators

Q. Name the plasma-derived mediators of inflammation.

Chemical mediators derived from plasma proteins belong to three interrelated systems:

1. Complement
2. Kinin
3. Clotting systems.

Complement System

Q. What are the three methods of complement activation and its effector function in acute inflammation?

The complement system is a group of plasma proteins synthesized in the liver, and are numbered C1 to C9.

Pathways of complement system activation (Fig. 2.8): The decisive step in complement activation is the proteolysis of the third component, C3.

Cleavage of C3 can occur by any one of three pathways:

1. **Classical pathway:** It is activated by **antigen-antibody** (Ag-Ab) complexes.
2. **Alternative pathway:** It is triggered by microbial surface molecules (e.g. endotoxin, or LPS), complex polysaccharides, cobra venom, and other substances, **in the absence of antibody**.
3. **Lectin pathway:** It directly activates C1 when plasma mannose-binding lectin binds to mannose on microbes.

C3 is the complement component that can be activated by (1) classical (Ag+Ab complexes), (2) alternate pathway and (3) lectin pathway.

C1 inhibitor: Blocks activation of C1. Inherited deficiency of C1 inhibitor is associated with hereditary angioedema (edema at multiple sites including the larynx).

Functions of Complement

Anti-infective functions:

1. **Leukocyte activation, adhesion and chemotaxis:** C5a causes **leukocyte activation, adhesion** and C3a and C5a are **powerful chemotactic** agents for neutrophils, monocytes, eosinophils, and basophils.
2. **Opsonization and promote phagocytosis:** C3b and its cleavage product iC3b (inactive C3b) act as opsonins and promote phagocytosis by neutrophils and macrophages through surface receptors for these complement fragments.

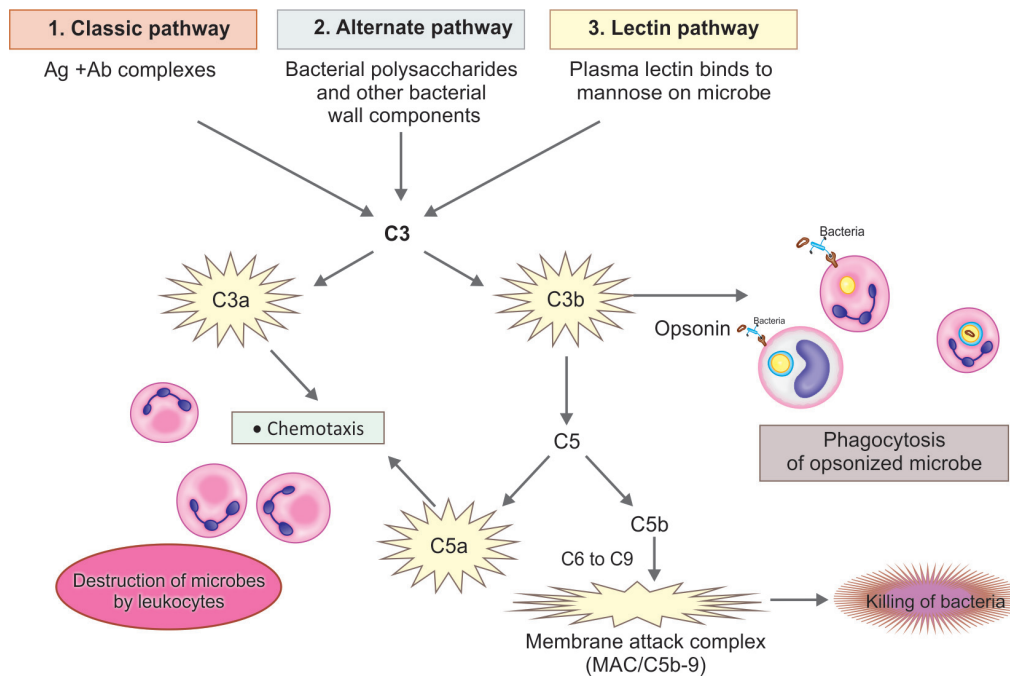


Fig. 2.8: Different pathways of activation and functions of the complement system. All pathways of activation lead to cleavage of C3

Activation of classic pathway is associated with:

1. Decreased levels of C1, C2, C4 and C3
2. Normal levels of factor B.

Activation of alternate complement pathway is associated with:

1. Decreased levels of Factor B and C3
2. Normal levels of C1, C2, and C4.

IgM and IgG (IgM>IgG): Responsible for activation of classical complement pathway.

IgA: Responsible for activation of alternate complement pathway.

Deficiency of C2:

1. Most common complement deficiency.
2. Associated with Streptococcal septicemia and lupus like syndrome in children.

3. **Cell and bacterial lysis:** The deposition of the MAC (C5b-C9) on cells creates pores, which allow water and ions to enter into the cells and results in death (lysis) of the cells and bacteria.
4. **Increased vascular permeability:** C3a, C5a complement components stimulate histamine release from mast cells and thus increase vascular permeability and cause vasodilation. They are called anaphylatoxins, because their actions are similar to mast cell mediators involved in anaphylaxis.
5. **Activation of AA:** C5a activates the lipoxygenase pathway of AA metabolism in neutrophils and monocytes, thereby causing release of more chemical mediators.

Interplay between innate and adaptive immune system:

- Defense against microbes through innate and adaptive immunity.

Other functions:

- Clearance of:
 - Immune complexes (C1q, C3)
 - Apoptotic cells (C1q, C3).

Complement components can cause chemotaxis (C3a C5a), opsonization (C3b) and killing (MAC) and increased vascular permeability.

Critical step in complement system : Activation of C3.

C3a and C5a are called anaphylotoxins, because their actions are similar to mast cell mediators involved in anaphylaxis.

Activation of complement is controlled by cell-associated and circulating regulatory proteins. These include: C1 inhibitor, decay-accelerating factor (DAF), and factor H.

Coagulation and Kinin Systems

Inflammation and clotting system are intertwined with each other.

Activated Hageman factor (factor XIIa) activate the four systems involved in the inflammatory response (Fig. 2.9).

1. **Activation of fibrinolytic system:** Factor XIIa stimulates fibrinolytic system by converting plasminogen to plasmin. The role of fibrinolytic system in inflammation are:
 - **Activation of complement system.**

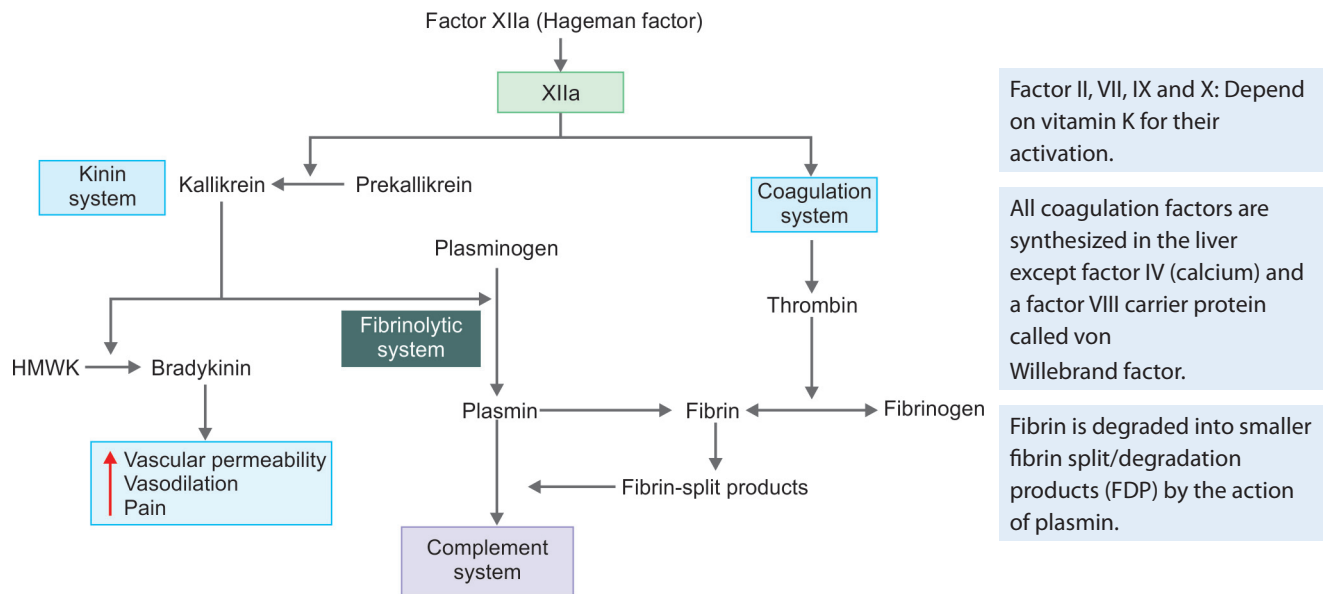


Fig. 2.9: Interrelationships between the four plasma-derived chemical mediator systems. Activation of factor XII (Hageman factor) is a key event leading to conversion of plasminogen to plasmin, resulting in generation of fibrin split products and active complement products. Activation of kallikrein produces kinins and activation of the coagulation system results in fibrin formation

Abbreviation: HMWK, high-molecular-weight kininogen

- **Fibrin split products:** Plasmin degrades fibrin to form fibrin split products, which may **increase vascular permeability**.

2. Activation of the Kinin system

3. **Activation of the alternative complement pathway:** Factor XIIa can activate alternate complement pathway.

4. **Activation of the coagulation system:** Factor XIIa activates coagulation system and form thrombin, which has inflammatory properties.

Activated factor XII (XIIa) triggers activation of:

1. Coagulation system
2. Kinin system
3. Complement system
4. Fibrinolytic system.

Kinins System

Kinins are vasoactive peptides derived from plasma proteins.

- **Mechanism of production:** Factor XIIa converts prekallikrein to kallikrein, which in turn cleaves high-molecular-weight kininogen to produce bradykinin.
- **Actions of bradykinin:**
 - Increases vascular permeability
 - Pain when injected into the skin.
- **Actions of kallikrein:**
 - Potent activator of Hageman factor

- Chemotactic activity: Directly converts C5 to the chemoattractant product C5a.

Most important mediators involved in acute inflammation are summarized in Table 2.9.

Bradykinin mediates increased vascular permeability and pain.

Cells of Inflammation

Leukocytes are the major cells involved in inflammation. These include neutrophils, lymphocytes (T and B), monocytes, macrophages, eosinophils, mast cells and basophils.

Neutrophils

Polymorphonuclear neutrophils (PMNs) are characteristic and predominant cells of acute inflammation. They are stored in bone marrow and circulate in the blood (constitute 40–75% of circulating leukocytes). During inflammation, they rapidly accumulate at sites of injury or infection. PMNs have granular cytoplasm and a 2- to 4-lobed nucleus. Polymorphonuclear neutrophil phagocytose the invading microbes and dead tissue. They undergo apoptosis, mainly during the resolution phase of acute inflammation. However, they can damage the tissues such as basement membrane and small blood vessels in immunologic cell injury. In chronic bacterial infection of bone (osteomyelitis), a neutrophilic exudate may be

Q. Write short note on role of different mediators in different reactions of inflammation.

TABLE 2.9: Important mediators involved in acute inflammation

Action of the mediator	Name of the mediator	Source of the mediator
Vasodilation	Prostaglandins	Mast cells, all leukocytes
	Nitric oxide	Endothelium, macrophages
	Histamine	Mast cells, basophils, platelets
Increased vascular permeability	Histamine	
	Serotonin	Platelets
	C3a and C5a (liberate vasoactive amines from mast cells, other cells)	Plasma (produced in liver)
	Bradykinin	
	Leukotrienes C ₄ , D ₄ , E ₄	Mast cells, all leukocytes
	Platelet-activating factor (PAF)	All leukocytes, endothelial cell
	Neuropeptides (substance P)	Leukocytes, nerve fibers
Chemotaxis and leukocyte activation	Cytokines (TNF, IL-1, IL-6)	Macrophages, lymphocytes, endothelial cells, mast cells
	Chemokines	Leukocytes, activated macrophages
	C3a, C5a	Plasma (produced in the liver)
	Leukotriene B ₄	Mast cells, leukocytes
	Bacterial products (e.g. <i>N</i> -formyl methyl peptides)	Bacteria
Fever	IL-1	Macrophages, endothelial cells, mast cells
	TNF	
	Prostaglandins	
Pain	Prostaglandins	
	Bradykinin	Plasma protein
Tissue damage	Lysosomal enzymes	Leukocytes
	Reactive oxygen species	
	Nitric oxide	Endothelium, macrophage

Abbreviations: IL-1, interleukin-1; IL-6, interleukin-6; TNF, tumor necrosis factor.

observed for months and this pattern of inflammation is termed as acute on chronic.

Eosinophils

Eosinophils circulate in blood (constitute 1–6% of circulating leukocytes) and are recruited to tissue mainly in immune reactions mediated by IgE and in parasitic infections. There are recruited by specific chemokines (e.g. eotaxin) derived from leukocytes and epithelial cells. Eosinophil granules contain major basic protein (a highly cationic protein) which is toxic to parasites as well as leukotrienes, PAF, acid phosphatase and peroxidase. However, they produce to tissue damage in IgE-mediated immune reactions (e.g. allergies and asthma).

Major basic protein is present in eosinophils and is toxic to parasites.

Mast cells

They are widely distributed in connective tissues. They participate in both acute and chronic inflammatory reactions. Mast cells have surface receptor (FcεRI) which can bind with the Fc portion of IgE antibody. In immediate hypersensitivity reactions, IgE antibodies bound to mast cells recognize antigen/allergen and they degranulate. This results in release of mediators, such as histamine and prostaglandins. This occurs during allergic reactions to foods, insect venom, or drugs. Sometimes, it may have catastrophic results (e.g. anaphylactic shock).

Basophils

Basophils are the least common leukocyte in the blood (about 1%). They can migrate into tissue to participate in immunologic responses. They are functionally similar to mast cells and present in all supporting tissues. They play an important role in regulation of vascular permeability and bronchial smooth muscle contraction especially in type I hypersensitivity reactions. Mast cells are found in connective tissues (especially on lung and gastrointestinal mucosal surfaces, in the dermis and in the microvasculature).

Lymphocytes

Lymphocytes constitute about 20–45% of circulating leukocytes in adults. They are also present in large numbers in spleen, thymus, lymph nodes, and mucosa-associated lymphoid tissue (MALT). There are two types of lymphocytes namely B and T lymphocytes. They are discussed in detail in pages 74 and 125.

Plasma Cells

They have an eccentric nucleus with a paranuclear hof/clearing. The nuclear chromatin has a cart-wheel pattern. They synthesize antibody and are normally not present in peripheral blood. They are increased in chronic inflammations (e.g. syphilis, rheumatoid arthritis, tuberculosis), hypersensitivity states and multiple myeloma.

Macrophages (Discussed in Page 68)

OUTCOMES OF ACUTE INFLAMMATION (FIG. 2.10)

Q. Write short note on outcomes of acute inflammation.

- **Resolution:** Complete return of tissue architecture to normal following acute inflammation. It occurs:
 - When the injury is limited or short-lived

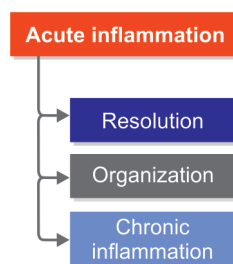


Fig. 2.10: Outcomes of acute inflammation: 1. resolution, 2. organization (healing by fibrosis and scarring), or 3. chronic inflammation

- With no or minimal tissue damage
- When injured tissue is capable of regeneration.
- **Organization/healing by fibrosis:** Process of **replacement of dead tissue by living tissue**, which matures to form scar tissue is known as **organization**. It occurs:
 - When there is plenty of fibrin exudation in tissue or serous cavities (pleura, peritoneum) which cannot be removed or cleared.
 - In presence of with significant tissue destruction.
 - With inflammation in tissues incapable of regeneration.

This process involves growing of connective tissue into the area of tissue damage or exudate, and is converted into a mass of fibrous tissue (scar).

- **Abscess:** Localized collection of pus is called abscess. If the area of acute inflammation is walled off by inflammatory cells and fibrosis, neutrophil products destroy the tissue and form an abscess.
- **Progression to chronic inflammation:** Chronic inflammation may follow acute inflammation, or it may be chronic from the beginning itself. Acute progress to chronic when the acute inflammatory response cannot be resolved. This may be due to:
 - Persistence of the injurious agent or
 - Abnormality in the process of healing.

Examples:

 - ♦ Bacterial infection of the lung may begin as acute inflammation (pneumonia). But when it fails to resolve, it can cause extensive tissue destruction and form a cavity with chronic inflammation known as lung abscess.
 - ♦ Acute osteomyelitis if not treated properly may progress to chronic osteomyelitis.
 - ♦ Chronic inflammation with a persisting stimulus results in peptic ulcer of the duodenum or stomach, which may persist for months or years.

MORPHOLOGICAL TYPES/PATTERNS OF ACUTE INFLAMMATION

Q. Write short note on morphological types/patterns of acute inflammatory reaction with suitable examples.

Gross and microscopic appearances can often provide clues about the cause.

Serous Inflammation

- Characterized by marked outpouring of a thin serous fluid.

- Serous exudate or effusion is yellow, straw-like in color and microscopically shows either few or no cells.
- **Example:**
 - Skin blister formed in burn or viral infection.
 - Inflammation of synovium (synovitis).
 - Pleural effusion as a complication of lobar pneumonia.

Effusion: Accumulation of fluid in serous cavities (peritoneal, pleural, and pericardial).

Fibrinous Inflammation

- **Marked increase in vascular permeability** leads to escape of large molecules like fibrinogen from the lumen of the vessel into the extravascular space and forms fibrin. The **exudate rich in fibrin** is called fibrinous exudate.
- A fibrinous exudate is mostly observed with inflammation in the lining of body cavities, such as the meninges, pericardium and pleura. When a fibrinous exudate develops on a serosal surface, such as the pleura or pericardium, it is known as fibrinous pleuritis or fibrinous pericarditis.
- Microscopically, fibrin appears as an eosinophilic or pink meshwork of threads or pink amorphous coagulum.
- For example, fibrinous pericarditis (refer Fig. 15.15) is seen in rheumatic fever and classically known as “**bread and butter**” pericarditis.

Suppurative or Purulent Inflammation: Abscess

- It is characterized by the production of large amounts of pus or purulent exudate.
- Microscopically, shows neutrophils, liquefactive necrosis, and edema fluid. Bacteria (e.g. staphylococci) which produce localized suppuration and are called as **pyogenic** (pus-producing) bacteria. For example, acute appendicitis.
- **Abscesses:** It is the **localized collections of purulent inflammatory exudates in a tissue**, an organ, or a confined space. Abscesses have a central necrotic focus (consisting of necrotic leukocytes and necrotic parenchymal cells) surrounded by a zone of preserved neutrophils. If pus accumulates in hollow organs or pleural cavity, it is known as empyema, e.g. Boil caused by *Staphylococcus aureus*.

Hemorrhagic Inflammation

- When inflammation is associated with severe vascular injury or deficiency of coagulation factors, it causes hemorrhagic inflammation, e.g. acute pancreatitis due to proteolytic destruction of vascular walls.

Catarrhal Inflammation

- Acute inflammation of a mucous membrane is accompanied by excessive secretion of mucus and the appearance is described as catarrhal, e.g. common cold.

Membranous Inflammation

- In this type, epithelium is covered by membrane consisting of fibrin, desquamated epithelial cells and inflammatory cells, e.g. pharyngitis or laryngitis due to *Corynebacterium diphtheria*.

Pseudomembranous Inflammation

- Superficial mucosal ulceration covered by sloughed mucosa, fibrin, mucus and inflammatory cells.
- For example, pseudomembranous colitis due to *Clostridium difficile* colonization of the bowel, usually following broad-spectrum antibiotic treatment.

Necrotizing (Gangrenous) Inflammation

The combination of necrosis and bacterial putrefaction is **gangrene** (refer Fig. 1.23), e.g. gangrenous appendicitis.

Ulcer

Q. Write short note on ulcer.

An ulcer is defined as a local defect, or excavation, of the surface of an organ or tissue. Common sites:

1. Mucosa of the mouth, stomach (e.g. peptic ulcer of the stomach or duodenum (refer Figs 18.6 and 18.7), intestines, or genitourinary tract.
2. Skin and subcutaneous tissue of the lower extremities (e.g. varicose ulcers).

Terminology

Bacteremia: It is defined as condition characterized by the presence of small number of bacteria in the blood. They cannot be detected by direct microscopic examination of blood and are detected by blood culture (e.g. typhoid infection caused by *Salmonella typhi*).

Septicemia: It is defined as the presence of rapidly multiplying, highly pathogenic bacteria in the blood (e.g. pyogenic cocci/bacilli). It is usually associated with systemic effects such as toxemia and neutrophilic leukocytosis.

Pyemia: It is the dissemination of small septic emboli in the blood which produce their effects at the site of their lodgment. Thus, it can lead to pyemic abscesses or septic infarcts.

Cellulitis: It is the term used for diffuse inflammation of the soft tissues due to organism produced from spreading effects of substances like hyaluronidase released by some bacteria.

SYSTEMIC EFFECTS OF INFLAMMATION

Q. Write short note on systemic effects of inflammation.

Systemic changes in acute inflammation are collectively known as acute-phase response, or the systemic inflammatory response syndrome (SIRS).

Causes: Due to cytokines produced by leukocytes, in response to infections or immune reactions. Most important cytokines are TNF, IL-1, and IL-6.

The clinical and pathologic changes of acute-phase response are:

1. Fever:

- **Pyrogens:** These are molecules that cause fever. It may be **exogenous** (bacterial products, like **LPS**), which stimulate leukocytes to release **endogenous pyrogens (cytokines such as IL-1 and TNF)**. The cytokines increase the enzymes cyclooxygenases resulting in conversion of AA into **prostaglandins**.
- Pyrogens and prostaglandins may act on hypothalamic thermoregulatory center causing fever.

Fever is produced by exogenous or endogenous pyrogens.

Q. Write short note on acute phase proteins/reactants.

2. **Raised plasma levels of acute-phase proteins:** These are plasma proteins synthesized in the liver and may be markedly raised in response to inflammatory stimuli.

- **Types of acute-phase proteins:** (1) **C-reactive protein (CRP)**, (2) **fibrinogen**, (3) **serum amyloid A (SAA) protein**. Their synthesis by hepatocytes is increased by cytokines, especially IL-6 (for CRP and fibrinogen) and IL-1 or TNF (for SAA).
- **Actions/functions:**
 - Many acute-phase proteins (CRP and SAA) bind to microbial cell walls and **may act as opsonins**.
 - Fibrinogen binds to red cells to form stacks (**rouleaux**) and responsible for **raised erythrocyte sedimentation rate (ESR)**.
 - During acute inflammation, acute-phase proteins have beneficial effects but prolonged production (especially SAA) like in chronic inflammation causes **secondary amyloidosis**.

Q. Write short note on C-reactive protein.

- **C-reactive protein (CRP)** is an acute phase reactant synthesized mainly by the liver. Its synthesis is stimulated by a number of inflammatory mediators (mainly by cytokines, e.g. IL-6) acting on liver cells. CRP augments the innate immune response by binding to microbial (bacteria) cell walls, may act as opsonins and activate the classical complement cascade. They also bind chromatin and helps in clearing necrotic cell nuclei.
- **Significance:** (1) Raised serum levels of CRP is a marker for increased risk of myocardial infarction in patients with coronary artery disease. Probably inflammation involving atherosclerotic plaques in the coronary arteries may predispose to thrombosis and subsequent myocardial infarction. (2) Plasma CRP is a strong, independent marker of risk for myocardial infarction, stroke, peripheral arterial disease, and sudden cardiac death, even in healthy individuals and (3) CRP is also a useful marker for assessing the effects of risk reduction measures, such as cessation of smoking, weight loss, exercise, and statins; each one of these reduce CRP levels.

Infections are associated with raised ESR.

Endogenous pyrogens: Cytokines (IL-1, TNF) stimulate production of prostaglandins in hypothalamus.

Exogenous pyrogen: Bacterial products (e.g. LPS).

NSAIDs inhibit prostaglandin synthesis and thereby reduce fever.

C-reactive protein (CRP): Marker of necrosis and disease activity.

3. Changes in the leukocytes:

- **Leukocytosis:** Total leukocyte count more than 11,000/ μ L are termed as leukocytosis. Common in inflammatory reactions, especially those caused by bacterial infections.
 - **Count:** May be increased up to 15,000 or 20,000 cells/ μ L. Sometimes, it may be extremely high reaching 40,000 to 100,000/ μ L associated with more immature neutrophils in the blood (shift to the left) and are called as **leukemoid reactions**, similar to the white cell counts found in leukemia. It is important to distinguish it from leukemia, which is a malignant disease.
 - **Cause:** It is due to increased release of leukocytes from the bone marrow caused by cytokines, including Colony stimulating factors (CSFs), TNF and IL-1.
 - **Bacterial infections** cause an increase in the blood neutrophil count known as **neutrophilia**.

- **Lymphocytosis:** It is seen in **viral infections** (e.g. Infectious mononucleosis, mumps, and German measles).
- **Eosinophilia:** It is seen in **bronchial asthma, allergy, and parasitic infestations**.
- **Leukopenia:** Decreased number of circulating white cells is associated with few infections like **typhoid fever and some viruses, rickettsia, and certain protozoa**.

Leukocytosis and neutrophilia are characteristically observed in **bacterial infections**.

Lymphocytosis: In viral infections, e.g. Infectious mononucleosis, mumps, and German measles.

Eosinophilia: In bronchial asthma, allergy, and parasitic infestations.

Leukopenia: Associated with few infections like typhoid fever and some viruses, rickettsia, and certain protozoa.

4. **Other features of the acute-phase response:** It includes:
 - Increased pulse and blood pressure.
 - Anorexia and malaise, probably due to cytokines acting on brain cells.
 - In severe bacterial infections (sepsis) cytokines (mainly TNF and IL-1) may be produced in large quantities and can result in disseminated intravascular coagulation and cardiovascular failure.

Polyclonal gammopathy: Indicates chronic inflammation.

Wound Healing

INTRODUCTION

Q. Define the term healing, regeneration and repair.

Injury to cells and tissues **results in loss of cells and tissues**. It sets in inflammation (restrict the tissue damage) and initiate replacement of lost tissue by living tissue.

Healing

Definition: Healing is a process of replacement of dead tissue by living tissue.

It can be broadly divided into regeneration and repair.

1. Regeneration:

Definition: Regeneration is a process in which **lost tissue is replaced by tissue of similar type**. It results in the complete restoration of lost or damaged tissue by proliferation of residual uninjured cells and replacement from stem cells.

2. Repair:

Definition: Repair is defined as a process in which **lost/damaged tissue is replaced by fibrous tissue or scar**.

Replacement of lost tissue may occur by regeneration with complete restoration or by replacement by connective tissue to form scar.

Most often healing occurs by a combination of regeneration and repair.

Repair is a healing process, but it may itself cause tissue dysfunction (e.g. in pathogenesis of atherosclerosis).

Factors Deciding the Pattern of Healing

The proportion of regeneration and repair process in healing depends on:

Proliferative Capacity of the Tissue

According to proliferative capacity of the cells, the tissues of the body can be divided into three groups:

1. **Labile (continuously dividing) tissues:** The cells of labile tissues proliferate throughout life, replacing the lost cells from stem cells. Examples:
 - Hematopoietic cells of the bone marrow
 - Surface epithelia of the skin, oral cavity, vagina, and cervix
 - Columnar epithelium of the gastrointestinal tract and uterus.

Labile tissues with regenerative capacity

- Hematopoietic cells
- Epithelium of skin and gastrointestinal (GI) tract.

2. **Stable (quiescent) tissues:** Cells of stable tissue normally do not proliferate; but can **proliferate in response to injury or loss of tissue**. Examples:

- **Parenchymal cells** of liver, kidneys, and pancreas
- **Mesenchymal cells:** Fibroblasts, vascular endothelial cells, smooth muscle cells, chondrocytes, and osteocytes.

Stable tissues: Proliferate in response to injury or loss of tissue, e.g. parenchymal cells of liver and kidney.

3. **Permanent (nondividing) tissues:** Cells of these tissues **cannot proliferate after birth**. In these tissues, repair is by scar formation. Example:

- **Neurons:** Damaged neurons are replaced by the proliferation of the **glial cells**
- **Skeletal muscle** cells
- **Cardiac muscle** cells.

However, limited stem cell replication and differentiation can occur in some areas of the adult brain, and heart muscle cells can proliferate after myocardial necrosis.

Permanent tissues: Cells cannot proliferate after birth, e.g. neurons, cardiac muscle.

Extent of Tissue Injury

- **Mild and short duration:** The damaged tissue is healed by regeneration without significant scarring.
- **Severe and chronic:** Healing occurs by fibrous tissue forming scar.
 - Severe tissue injury damages both parenchymal cells and the extracellular matrix (ECM) framework
 - Chronic inflammation.

STEM CELLS

Q. Write short note on stem cells.

Definition: Stem cells are characterized by their **ability of self-renewal and capacity to generate differentiated cell lineages**.

Properties

1. **Self-renewal capacity** and capacity to generate differentiated cell lineages.
2. **Asymmetric replication:** This is characterized by division of stem cell into two cells:
 - One daughter cell which gives rise to mature cells
 - Other cell remains as undifferentiated stem cell which retains the self-renewal capacity.

Stem cell:

- Self-renewal capacity
- It is an dormant phase of cell cycle
- Asymmetric replication
- Capacity to generate differentiated cell lineages.

Types

1. **Embryonic stem cells:** During development of embryo, the blastocysts contain **undifferentiated pluripotent**

stem cells, which are called as embryonic stem cells or ES cells. These cells **can form cells of all three germ cell layers**.

- Normal function: To give rise to all cells of the body.
2. **Adult (somatic) stem cells:** Adult stem cells are less undifferentiated than ES cells found in adults. They are found among differentiated cells within a tissue. They have **more limited capacity to generate different cell types than ES cells**. They usually differentiate into particular tissue.
 - Normal function: Tissue homeostasis.
 3. **Induced pluripotent stem cells (iPS cells):** This is achieved by transferring the nucleus of adult cells to an enucleated oocyte.
 - **Use:** For **therapeutic cloning** in the treatment of human diseases.

Types of stem cells: (1) embryonal, (2) adult and (3) induced

Embryonal cells are pluripotent cells capable of forming cells of all three germ cell layers.

Sites of Stem Cells

Stem cells reside in special microenvironments called niches.

1. **Bone marrow:** It contains two types of stem cells
 - **Hematopoietic stem cells (HSCs):**
 - They can generate all of the blood cell lineages, and are used for the treatment of hematologic diseases.
 - They can be collected directly from the bone marrow, from umbilical cord blood, and from the peripheral blood.
 - **Marrow stromal cells (MSCs):** They can generate chondrocytes, osteoblasts, adipocytes, myoblasts, and endothelial cell precursors depending on the tissue to which they migrate.

Stem cells: Used in bone marrow transplantation in the treatment of various types of leukemia and lymphoma.

2. **Intestinal epithelium:** Stem cells may be located immediately above Paneth cells in the small intestine or at the base of the crypt in the colon.
3. **Liver:** The liver contains stem cell in the canals of Hering, which are capable of differentiating into hepatocytes and biliary cells.
4. **Cornea:** Located in the limbus region between the conjunctiva and the cornea.
5. **Skin:** Located in the bulge area of the hair follicle, in the sebaceous glands, and in the lower layer of the epidermis.

CELL CYCLE AND CELL PROLIFERATION

- Inflammation is the primary response of living tissue to injury.
- With inflammation, there will be damage or loss of tissue, which has to be replaced by living tissue. This replacement is done by transient increase in cellularity due to proliferation of cells by either regeneration and/or by repair.
- Proliferation of cells is characterized by DNA replication and mitosis. The sequence of events that control DNA replication and mitosis is known as the **cell cycle**.

Definition of cell cycle: Cell proliferation is a regulated process, which involves activators and inhibitors, as well as checkpoints.

Phases of Cell Cycle (Fig. 3.1)

- G₁ (presynthetic)
- S (DNA synthesis)
- G₂ (premitotic)
- M (mitotic) phase.

Checkpoints: They check whether there is any damage to DNA and chromosomes in the replicating cells. These checkpoints make sure that only normal cells complete replication. There are two checkpoints:

1. G₁/S checkpoint monitors the integrity of DNA before replication.
2. G₂/M checkpoint checks DNA after replication and monitors whether the cell can safely enter mitosis.

Proliferation of cells occurs when quiescent cells enter the cell cycle.

Growth Factors

Q. Write short note on growth factors.

Definition: Growth factors stimulate the survival and proliferation of particular cells and most of them are proteins.

Mechanism of action: Growth factors induce cell proliferation by **binding to specific receptors**, and deliver positive growth signals to the target cells. These signals stimulate the **expression of genes** whose products have several functions which include:

- Activation of cell cycle
- Relieve blocks which prevent cell cycle progression
- Prevention of apoptosis
- Increases the synthesis of cellular proteins.

Growth factors: Multiple effects and include cell proliferation, survival, migration, contractility, differentiation, and angiogenesis.

Various growth factors involved in wound healing and regeneration are listed in Table 3.1.

Signaling Mechanisms of Growth Factor Receptors

Q. Write short note on different types of signaling.

The **receptor-mediated signal transduction** process is activated by the binding of ligands (e.g. growth factors and cytokines) to specific receptors. Receptor activation leads to expression of specific genes.

Modes of signaling (Figs 3.2A to C): Depending on the source of the ligand and the location of its corresponding

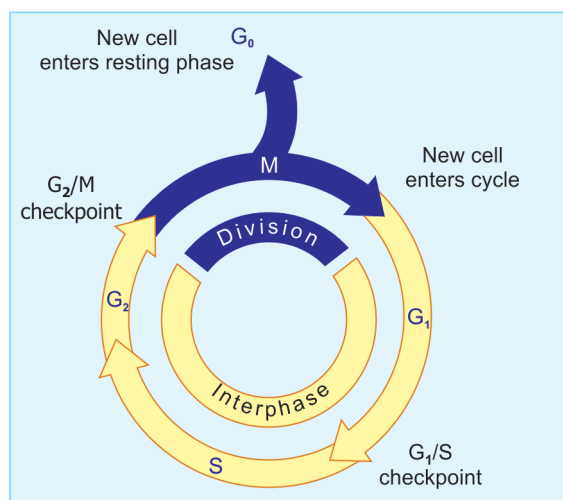


Fig. 3.1: Cell cycle showing different phases (G₀, G₁, G₂, S, and M). Cells from labile tissues (e.g. epidermis) may remain in cycle continuously; stable cells (e.g. liver cells) are quiescent but can enter the cell cycle; permanent cells (e.g. neurons) have lost the capacity to proliferate and cell cycle arrests in the G₁ phase or exit the cycle and are in G₀ phase

Two checkpoints in cell cycle:

1. G₁/S checkpoint
2. G₂/M checkpoint.

Cell cycle is a tightly regulated process and has checkpoint controls which prevent the proliferation of abnormal cells.

Major action of growth factors is to stimulate genes that control growth. Many of them are called as proto-oncogenes.

Quiescent cells, which have not entered the cell cycle, are in the G₀ state.

G₁ phase: Most variable phase in cell cycle.

G₁ to S phase: Most critical phase in the cell cycle.

TABLE 3.1: List of growth factors and cytokines involved in wound healing and regeneration

A. GROWTH FACTOR		
Type of growth factor	Receptor	Functions
EGF family 1. Epidermal growth α (EGF) 2. Transforming growth factor α (TGF- α)	EGFR 1. EGFR1 (ERBB1) 2. ERBB2 (HER-2 or HER2/Neu)	Keratinocyte migration Formation of granulation tissue
Hepatocyte growth factor/scatter factor (HGF/SF)	c-MET	Proliferation of epithelial cells, hepatocytes, and endothelial cells Increases cell motility Keratinocyte replication
Platelet-derived growth factor (PDGF) • Isoforms A, B, C, D	PDGFR α and β	Chemotaxis and activation of PMNs, macrophages Activation and proliferation of fibroblasts, smooth muscle cells and endothelial cells Stimulates production of ECM
Vascular endothelial cell growth factor (VEGF) • Isoforms A, B, C, D	VEGFR-1, VEGFR-2, and VEGFR-3	Increases vascular permeability; Mitogenic for endothelial cells Angiogenesis
Fibroblast growth factor (FGF) family Keratinocyte growth factor (FGF-7)	FGFRs 1–4	Wound repair-epithelialization [FGF-2 and KGF (FGF-7)] Angiogenesis (FGF-2)
Transforming growth factor β (TGF- β) and related growth factors TGF- β isoforms (TGF- β 1, TGF- β 2, TGF- β 3)	TGF- β receptors (types I and II)	Growth inhibitor for most epithelial cells Potent fibrogenic agent Strong anti-inflammatory effect
B. CYTOKINES		
• Tumor necrosis factor (TNF) and IL-1 participate in wound healing • TNF and IL-6 are involved in liver regeneration	TNF receptor (TNFR), or death receptor, for TNF, Interleukin-1 receptor (IL-1R) for IL-1 and interleukin 6 receptor (IL-6R) also known as CD126 (Cluster of differentiation 126) for IL6	TNF activates macrophages; regulates other cytokines and has multiple functions

receptors (i.e. in the same, adjacent, or distant cells), the modes of signaling can be divided into three types:

1. **Autocrine signaling:**

- Signaling molecules **act on the cells which secretes them.**
- Examples: Liver regeneration, proliferation of antigen-stimulated lymphocytes, tumors.

2. **Paracrine signaling:**

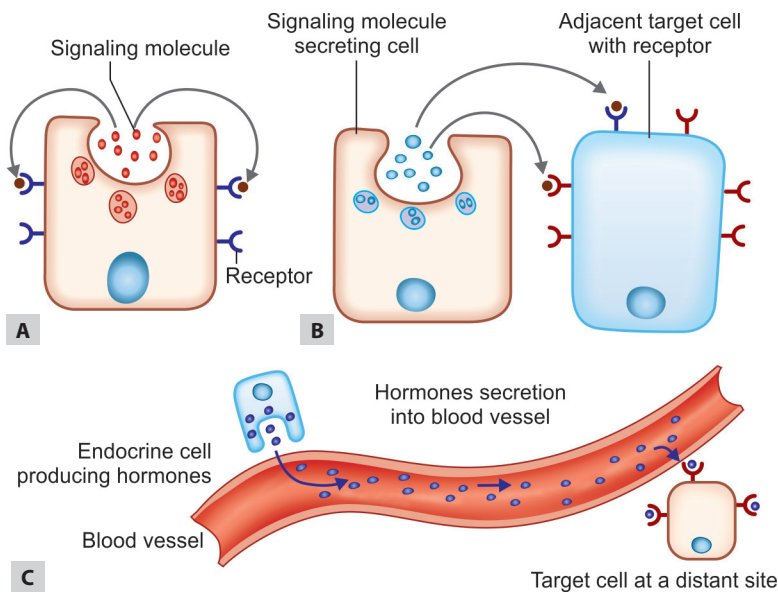
- Signaling molecule is produced by one cell type, that acts on adjacent target cells (usually of a different type) which expresses the appropriate receptor.
- Example: **Healing by repair:** Factor produced by macrophage (one cell type) has growth effect on fibroblast (adjacent target cells of different type).

3. **Endocrine signaling:**

- **Hormones:** These are produced by cells of endocrine organs, are usually carried by the blood and act on **target cells that are at a distant from the site of its synthesis.**

HEALING BY REPAIR, SCAR FORMATION AND FIBROSIS

Healing may be either by **regeneration or repair or combination of both.** With mild and transient injury, there is regeneration. If the tissue injury or damage persists, inflammation becomes chronic, resulting in excessive deposition of connective tissue known as **fibrosis** (repair).



Figs 3.2A to C: Modes of signaling: (A) Autocrine; (B) Paracrine and (C) Endocrine signaling.

NF κ B: Considered as master switch to the nucleus.

Growth factors: Produced transiently in response to stimuli and act by binding to specific receptors.

Growth factors: Some of them may also act at distant site (e.g. HGF).

Some tumors may produce excessive growth factors and/or their receptors resulting in their uncontrolled proliferation.

Growth factors act in autocrine, paracrine or endocrine signaling.

Cytokines: Several cytokines in inflammation may also act through endocrine signaling.

In most healing processes, a combination of repair and regeneration occurs.

Steps in Healing by Repair (Scar Formation)

Q. Write short note on steps of wound healing.

Inflammation

Whenever there is tissue injury, inflammatory reaction begins which tries to limit the damage and remove the injured tissue. At the same time, it also promotes the deposition of ECM components at the site of injury and stimulates angiogenesis.

Angiogenesis

Q. Write short note on angiogenesis in repair.

Definition: Angiogenesis is the process of formation of new blood vessels from existing vessels.

Steps in angiogenesis (Figs 3.3A to D):

- **Vasodilatation** in response to nitric oxide and **increased permeability of the pre-existing vessel** due to VEGF.
- **Separation of pericytes** from the abluminal surface of blood vessel. Breakdown of the basement membrane to facilitate formation of a vessel sprout.
- **Migration and proliferation of endothelial cells** toward the site of injury fibroblast growth factors (FGFs), mainly FGF-2.
- **Maturation of endothelial cells** and remodeling into capillary sprouts/tubes.

- **Formation of mature vessel:** It involves recruitment of pericytes and smooth muscle cells to form the periendothelial layer.
- **Suppression of endothelial proliferation** and migration, and deposition of basement membrane.

Angiogenesis is the process of formation of new blood vessels from existing vessels.

Growth factors involved in angiogenesis: Most important are vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF-2).

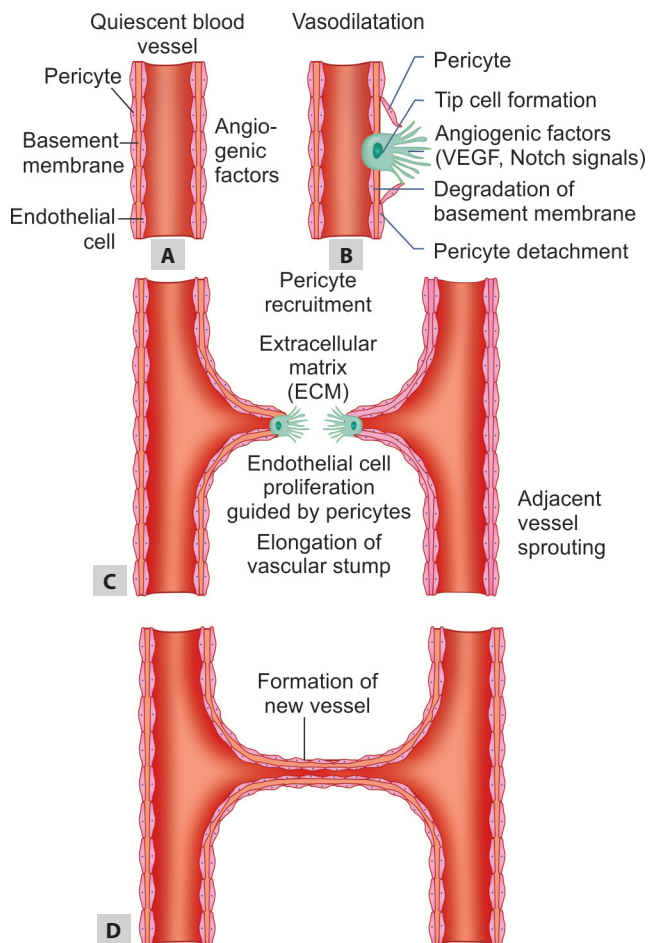
Formation of Granulation Tissue

Q. Write short note on granulation tissue.

The first 24 to 72 hours of the repair process begins with proliferation of fibroblasts and vascular endothelial cells. It forms a specialized type of tissue known as **granulation tissue**, which is a **hallmark/characteristic of tissue repair**.

The term granulation tissue is derived from its pink, soft, granular appearance on the surface of healing wounds.

- **Microscopy (Figs 3.4A and B):** Its characteristic features are:
 - Presence of **new small blood vessels** (angiogenesis): The new blood vessels are **leaky**, which allow the passage of plasma proteins and fluid into the extravascular space, which is responsible for edema often seen in granulation tissue.
 - **Proliferation of fibroblasts.**



Figs 3.3A to D: Mechanism of angiogenesis. (A) Normal blood vessel; (B) Vasodilation: First, pericytes separate followed by mobilization and proliferation of endothelial cells from the existing vessel to form capillary sprouts towards the site of injury (angiogenic stimuli); (C) Endothelial cells proliferate, loosely following each other, and are presumably guided by pericytes. Maturation of vessel (stabilization) involves the recruitment of pericytes and smooth muscle cells to form the periendothelial layer; (D) Finally, blood-vessel sprouts will fuse with other sprouts to build new circulatory systems

Microscopically, granulation tissue consists of:

1. New small blood vessels
2. Fibroblasts.

- **Amount** of granulation tissue formed depends on the:
 - **Size of the tissue deficit** created by the wound
 - **Intensity of inflammation.**

Scar Formation

- The leukocytes, edema, and angiogenesis disappear, accomplished by the **increased accumulation of collagen**. The **granulation tissue scaffolding is converted into a pale, avascular scar**.

Granulation tissue is essential for repair.

- **Components of scar:** It is composed of spindle-shaped fibroblasts, dense collagen, fragments of elastic tissue, and other ECM components.
- **By the end of the first month**, the scar consists of acellular connective tissue without inflammatory infiltrate.

Connective Tissue Remodeling

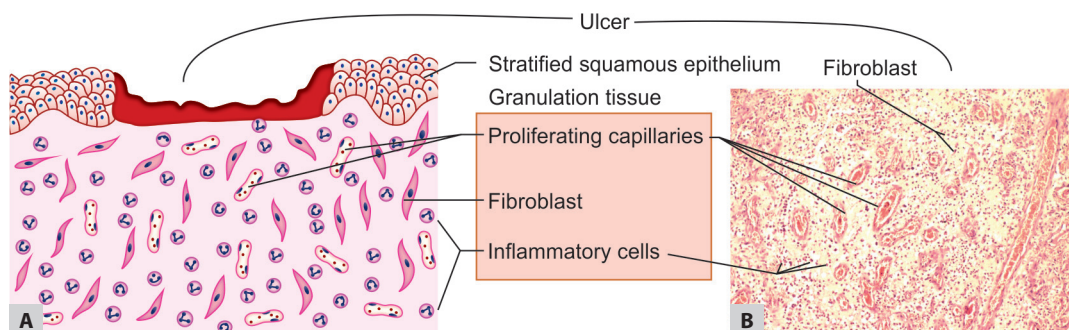
- Remodeling of the connective tissue framework is an important feature. It is the long-lasting phase of tissue repair.
- Remodeling indicates that the equilibrium/balance between ECM synthesis (collagen deposition) and degradation has been restored.

Role of Macrophages in Repair

Q. Write short note on role of macrophages in inflammation/repair.

Macrophages are important cells involved in repair. Their functions in repair include:

- Clear the offending agents and dead tissue.



Figs 3.4A and B: Granulation tissue consisting of numerous blood vessels, fibroblasts and edema. (A) Diagrammatic; (B) Hematoxylin and eosin (H and E)

- Provide growth factors for the proliferation of various cells.
- Secret cytokines that stimulate fibroblast proliferation and connective tissue synthesis and deposition.

CUTANEOUS WOUND HEALING

Q. Describe the healing of a clean surgical wound/healing by first intention.

Healing by Primary Union or by First Intention

Definition: Healing of a **clean, uninfected surgical incision** in the skin joined with surgical sutures is known as healing by **primary union or by first intention**.

Surgical incision causes **death of a minimum number of epithelial and connective tissue cells**. The **disruption of epithelial basement membrane continuity** is also **minimal**.

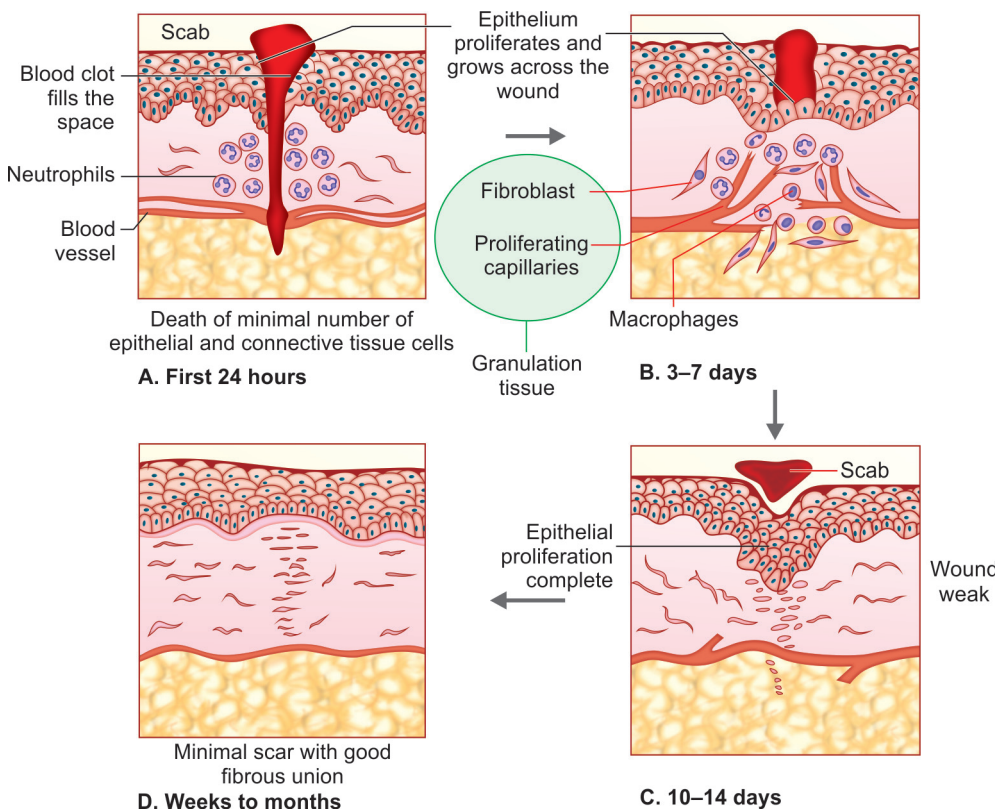
Re-epithelialization occurs by regeneration and there is a **relatively thin scar**. This is simplest type of cutaneous wound healing.

Stages in the Healing by First Intention

(Figs 3.5A to D)

• First 24 hours:

- **Formation of blood clot:** It is formed in the space between sutured margins. Blood clot contains not only trapped red cells but also fibrin, fibronectin and complement components. Clot stops bleeding and acts as a scaffold for migrating and proliferating cells. **Dehydration at the external surface of the clot** leads to formation of a **scab** over the wound.
- **Neutrophil infiltration:** Within 24 hours of wound, **neutrophils** appear at the margins of the incision. Neutrophils use the scaffold produced by the fibrin clot for its migration. They **release proteolytic enzymes** which clean out debris.



Wound healing: Neutrophils are the predominant cells during first 24 hours and are replaced by macrophages within 48 hours.

Early granulation tissue consists of type III and I collagen.

Factors which promote wound healing

- Clean wounds with closely apposed edges (sutured wound)
- No infection
- Good blood supply to the region
- Good nutrition including vitamin C
- Young age
- No metabolic abnormality
- Good circulatory status

Figs 3.5A to D: Healing by primary intention. (A) A wound with closely apposed edges and minimal tissue loss. The blood clots and fills the gap between the edges of the wound; (B) Epithelium at the edges proliferates. Minimal amount of granulation tissue is formed; (C) The epithelial proliferation is complete and the wound is weak; (D) Fibrosis with a small scar.

- **Epithelial changes:** At the cut edges of the wound, the **basal cells of the epidermis** begin to show **mitotic activity**. Epithelial cells from both the edges of wound proliferate and migration across the wound along the dermis.
- **Two days:**
 - **Neutrophils are replaced by macrophages.**
 - The **epithelial cells fuse in the midline below the surface scab** and epithelial continuity is re-established in the form of a thin continuous surface layer.
- **Three to seven days:**
 - **Granulation tissue** begins to invade incision space. It progressively grows into the **incision space/wound** and **fills the wound area** by 5–7 days. Collagen is progressively laid down.
 - **Surface epidermis** achieves its **normal thickness** and differentiation. It matures with surface keratinization.
 - Acute **inflammatory response** begins to **subside**.
- **Ten to fourteen:**
 - Leukocytic infiltration, edema, and angiogenesis disappear during the second week.
 - **Increased accumulation of collagen** and **regression of vascular channels**. The **granulation tissue scaffolding is converted into a pale, avascular scar**. Wound normally gains about 10% strength of normal skin. Further fibroblast proliferation occurs with collagen deposition.
- **Weeks to months:**
 - The **scar** appears as **acellular** connective tissue covered by intact epidermis and without inflammatory infiltrate.
 - Collagen deposition along the line of stress and **wound gradually achieves maximal 80% of tensile strength** of normal skin.

Healing by Secondary Union or by Second Intention

Q. Describe the mode of healing of wound by second intention.

Definition: When injury produces **large defects** on the skin surface with **extensive loss of cells and tissue**, the healing process is more complicated. Healing in such cutaneous wound is referred to as healing by **secondary union or by second intention**.

Basic mechanisms of healing by primary (first intention) and secondary (second intention) union are similar.

Features of Healing by Secondary Intention (Figs 3.6A to D)

- Larger wounds show **more exudate and necrotic tissue**. The **clot or scab** formed at the surface of wound is **large**. Full epithelialization of the wound surface is slow because of the larger gap.
- **Severe inflammatory reaction** because of larger defect and greater necrotic tissue.
- The **larger defect requires more** amount of (abundant) **granulation tissue**.
- Extensive deposition of collagen with substantial scar formation.
- **Wound contraction:** Wound contraction generally occurs in large surface wounds and is an important feature in healing by secondary union.

Wound contraction is an important feature of healing by secondary intention and is mediated by myofibroblasts.

Myofibroblasts of granulation tissue **have ultrastructural features of smooth muscle cells**. They contract in the wound tissue and are responsible for wound contraction.

Advantages of wound contraction:

- Decreases the gap between its dermal edges of the wound
- Reducing the wound surface area.

Wound Strength

Major portion of the connective tissue in repair is fibrillar collagens (mostly type I collagen) and are responsible for the development of strength in healing wounds.

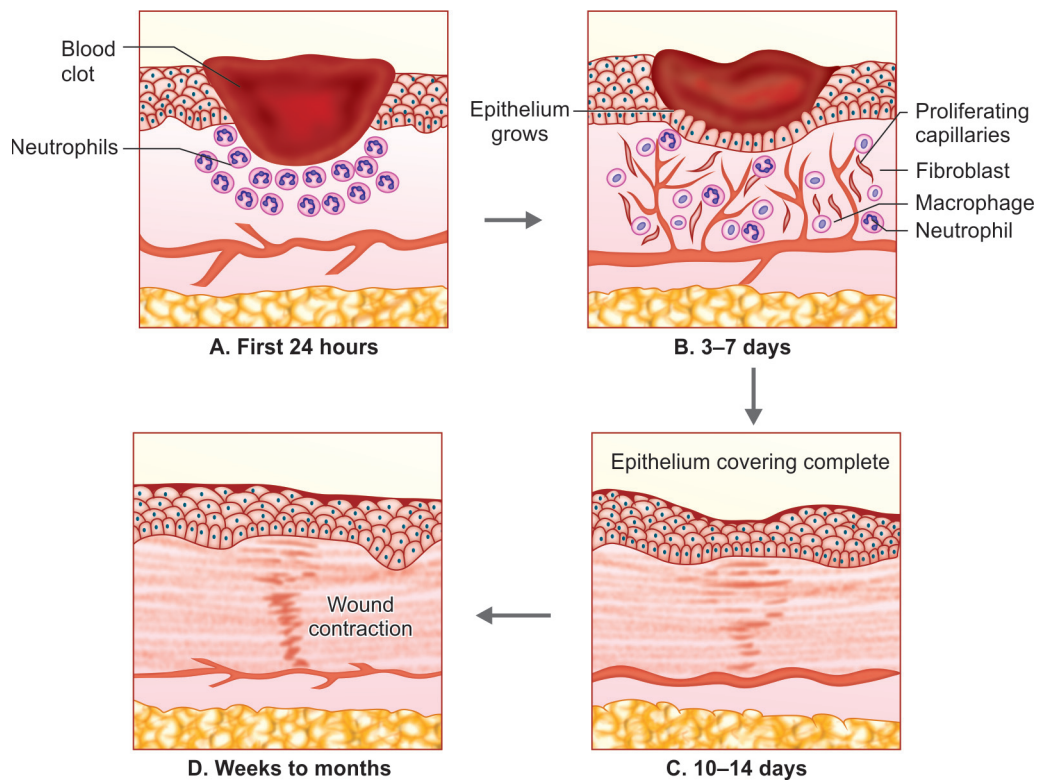
Time for a Skin Wound to Achieve its Maximal Strength

- **At the end of the first week:** When sutures are removed from an incisional surgical wound, **wound strength** is about **10%** that of normal unwounded skin.
- **Four weeks:** Wound strength quickly increases over the next 4 weeks, and then slows down.
- **Three months:** Wound strength **reaches a 70–80% of the tensile strength** of unwounded skin.

Wound strength:

- 10% after 1st week
- Rapidly increases during next 4 weeks
- 70% at the end of 3rd month.

Differences between healing by primary and secondary intention (Table 3.2).



Predominant collagen in adult skin: Type I.

Healing by secondary intention:

- Larger wound with extensive loss of tissue
- More exudate and necrotic tissue
- Wound contraction.

Factors which delay wound healing

- Infection
- Mechanical factors
- Foreign bodies
- Large wounds
- Wound over skin covering bone
- Poor blood supply
- Ionizing radiation
- Nutritional deficiency
- Old age
- Metabolic diseases
- Steroid use
- Hematological abnormalities

Figs 3.6A to D: Healing by secondary intention. (A) There is significant loss of tissue and the edges are far apart. Acute inflammation develops both at the edges and base; (B) The cell proliferation starts from the edges and large amount of granulation tissue is formed; (C) The wound is covered on the entire surface by the epithelium. The collagen fibers are deposited; (D) Granulation tissue is replaced by a large scar. There is significant wound contraction

Q. Tabulate the differences between healing by primary and secondary intention.

TABLE 3.2: Differences between healing by primary and secondary intention

Feature	Primary intention	Secondary intention
Nature of wound	Clean surgical wound	Unclean
Margins	Surgical clean margin	Irregular
Sutures	Used for apposition of margins	Cannot be used
Infection	Absent	May be infected
Amount of granulation tissue	Scanty at the incised gap and along suture track	Abundant and fill the gap
Outcome	Neat linear scar	Irregular contracted scar
Complications	Rare	Infection and suppuration

FACTORS THAT INFLUENCE WOUND HEALING

Q. List the factors that influence wound healing.

Q. List the factors which promote healing.

Q. List the factors which delay healing.

Local Factors

1. **Infection:** It is the single most important cause for delay in healing. Infection causes persistent tissue injury and inflammation.
2. **Mechanical factors:** Movement of wounded area may compress the blood vessels and separate the edges of the wound and can result in delayed healing.

Infection is the most common cause of delay in wound healing.

3. **Foreign bodies:** Unnecessary sutures or foreign bodies (fragments of steel, glass), or even bone can delay healing.
4. **Size and type of wound:** Small surgical incisional or other injuries heal quickly with less scar formation than

large excisional wounds or wounds caused by blunt trauma.

5. **Location of injury:** Wound over the skin covering bone with little intervening tissue prevents wound contraction (e.g. skin over the anterior tibia). The edges of skin lesions (e.g. burns) in such locations cannot be apposed.
6. **Blood supply:**
 - Wounds in areas with **good blood supply**, such as the face, heal **faster** than those with **poor blood supply**, such as the foot.
 - **Varicose veins** of the legs decrease the venous drainage and **can cause nonhealing ulceration**.
 - **Bed sores (decubitus ulcers)** result due to prolonged, localized, pressure, which diminishes both arterial and venous blood flow.
7. Ionizing radiation decreases repair process.
8. Complications may delay wound healing.

Systemic Factors

1. **Nutritional deficiencies:** Delays wound healing and these include:
 - Protein deficiency (e.g. malnutrition).
 - Vitamin C deficiency: Inhibits collagen synthesis and retard healing.
 - Trace elements: Copper and zinc deficiency.

Vitamin C:

1. Essential for synthesis of collagen
2. Antioxidant
3. Reducing agent.

Vitamin C deficiency:

1. Decreases cross-linking of trophecollagen
2. Decreases tensile strength of wound.
2. **Age:** Wound healing is rapid in young compared to in aged individuals.
3. **Metabolic status: Diabetes mellitus** is associated with delayed healing due to microangiopathy.
4. **Circulatory status: Inadequate blood supply** (due to arteriosclerosis) or venous abnormalities (e.g. varicose veins) that retard venous drainage, delay healing.

Zinc: Acts as a cofactor in collagenase.

5. **Hormones: Glucocorticoids have anti-inflammatory effects** and also inhibit collagen synthesis, thereby **impair wound healing**.
6. **Hematological abnormalities:** Quantitative or qualitative defects in neutrophils and bleeding disorders may slow the healing process.

COMPLICATIONS OF WOUND HEALING

Inadequate Granulation Tissue Formation

Q. Mention the complications of wound healing.

Inadequate formation of granulation tissue or a deficient scar formation can cause **wound dehiscence** and **ulceration**.

Wound dehiscence: Most common life-threatening complication of wound that develops after abdominal surgery.

1. **Dehiscence** (the wound splitting open) or rupture of a wound is most common life-threatening complication after abdominal surgery. It is due to increased abdominal pressure/mechanical stress on the abdominal wound from vomiting, coughing, or ileus.
2. **Ulceration:**
 - Wounds can ulcerate due to inadequate angiogenesis during healing. For example, wounds in the leg of patients with atherosclerotic peripheral vascular disease or varicose veins usually ulcerate.
 - Nonhealing wounds also develop in regions devoid of sensation. These **neuropathic or trophic ulcers** may be seen in diabetic peripheral neuropathy, nerve damage from leprosy.
3. **Incisional hernia** resulting from weak scars of the abdominal wall due to a defect caused by prior surgery. They are due to insufficient deposition of extracellular matrix or inadequate cross-linking in the collagen matrix.

Excessive Scar Formation

Excessive formation of the components of the repair process can result in:

1. **Hypertrophic scar:** The accumulation of excessive amounts of extracellular matrix, mostly collagen may give rise to a raised scar at the site of wound known as a hypertrophic scar. They usually develop after thermal or traumatic injury, which involves the deep layers of the dermis.
2. **Keloid:** If the scar tissue grows/progress beyond the boundaries of the original wound and does not regress, it is called a keloid. Thus, keloid is an exuberant scar that recurs with still larger keloid after surgical excision.

Keloids: More frequent among dark-skinned persons.

Keloids: Excessive deposition of type III collagen.

Keloid: One of the complication of wound healing characterized by excessive production of ECM.

3. Exuberant granulation:

- **Pyogenic granuloma or granuloma pyogenicum** (Fig. 3.7):
 - ◆ This consists of the localized formation of excessive amounts of granulation tissue.
 - ◆ Such exuberant granulation tissue projects above the level of the surrounding skin and prevents re-epithelialization. This mass formed is often named as **proud flesh**.

Proud flesh: Exuberant granulation tissue also known as pyogenic granuloma or granuloma pyogenicum.

Pyogenic granuloma: Excessive granulation must be removed for restoration of the continuity of the epithelium.

- **Desmoids or aggressive fibromatoses:**
 - ◆ Incisional scars or traumatic injuries may be followed by excessive proliferation of fibroblasts and other connective tissue elements.
 - ◆ They are known as desmoids, or aggressive fibromatoses, which may recur after excision.

Desmoid is an aggressive fibromatosis usually develops in the anterior abdominal wall.

Excessive Contraction

- A decrease in the size of a wound due to myofibroblasts is known as **contraction**.
- An exaggeration of this contraction is termed **contracture** and results in deformities of the wound and the surrounding tissues.
- Consequences of contractures:
 - **Compromise movements:** For example, contractures that follow severe burns can compromise the movement of the involved region (Fig. 3.8) and joint movements.
 - **Obstruction:** For example, in GI tract contracture (stricture) can cause intestinal obstruction.

Contracture: Exaggeration of wound contraction.

Common sites for contractures are palms, the soles and the anterior aspect of the thorax.

Others

1. **Infection** of wound by microbes.
2. **Epidermal cysts** can develop due to persistence of epithelial cells at the site of wound healing.
3. **Pigmentation** may develop due to either colored particle left in the wound or due to hemosiderin pigment.

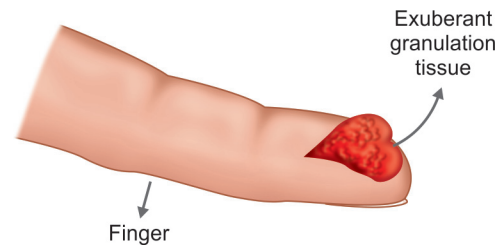


Fig. 3.7: Exuberant granulation tissue at the tip of the finger

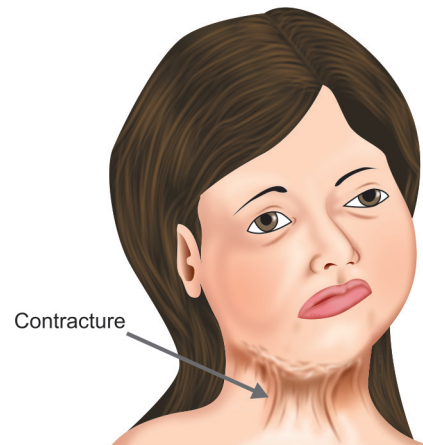


Fig. 3.8: Wound contracture—Severe contracture of a wound on the right side of neck, following burns

4. **Neoplasia:** For example, squamous cell carcinoma may develop in Marjolin's ulcer, which is the scar that follows burns in skin.

Fibrosis

TGF- β : Important fibrogenic agent.

- Normal wound healing is associated with deposition of collagen.
- The excessive deposition of collagen and other ECM components in a tissue is termed as fibrosis. It is usually observed in chronic inflammation.
- **TGF- β is an important fibrogenic agent.**
- Examples of disorders with **fibrosis**: Cirrhosis of liver, pneumoconioses, chronic pancreatitis and glomerulonephritis.

Complications of wound healing:

- A. Deficient scar formation
- B. Excessive formation of the repair components
- C. Formation of contractures
- D. Others.

Chronic Inflammation

INTRODUCTION

Definition: Chronic inflammation is defined as **inflammation of prolonged duration** (weeks or months) in which inflammation, tissue damage, and healing occurs at same time, in varying combinations.

Chronic inflammation may:

1. **Follow an acute inflammation**, which does not resolve (e.g. chronic osteomyelitis) or
2. **Begin as insidious, low-grade, chronic**, response without any acute inflammatory reaction.

Sequelae: Chronic inflammation can cause disabling tissue damage, e.g. rheumatoid arthritis, tuberculosis, and atherosclerosis.

Causes of Chronic Inflammation

Q. What are the causes of chronic inflammation?

1. **Persistent infections:** Microbes that are difficult to eradicate elicit delayed-type of hypersensitivity and produce chronic inflammation, e.g. mycobacteria, and certain viruses, fungi, and parasites. Some agents may cause a **distinct pattern of chronic inflammation** known as **granulomatous reaction**.
2. **Immune-mediated inflammatory (hypersensitivity) diseases:**
 - **Autoimmune diseases:** For example, rheumatoid arthritis.
 - **Allergic reactions:** For example, bronchial asthma.

- **Unregulated immune response:** For example, inflammatory bowel disease.

3. **Prolonged exposure to toxic injurious agents:**

- **Exogenous: Silica** is a nondegradable inanimate exogenous material. If persons are exposed to silica particles for long time, it causes an inflammatory lung disease called **silicosis**.
- **Endogenous: Atherosclerosis** is a disease of arterial intima, probably represents a chronic inflammatory process partly due to endogenous toxic plasma **lipid components**.

Most common cause of chronic inflammation: Persistent infection.

Causes of chronic inflammation:

1. Persistent infections
2. Immune-mediated inflammatory (hypersensitivity) diseases
3. Prolonged exposure to toxic agents.

Morphologic Features

Q. Mention the morphological/histological features cell of chronic inflammation.

Chronic inflammation is characterized by:

- **Mononuclear cells infiltrate:** Macrophages, lymphocytes, and plasma cells.
- **Tissue destruction** caused by the persistence of causative agent or by the inflammatory cells.
- **Healing by fibrosis.**

Chronic Inflammatory Cells and Mediators

Q. Write short note on cells of chronic inflammation.

Macrophages

Macrophage is the predominant cell in chronic inflammation.

Tissue macrophage: Derived from hematopoietic stem cells in the bone marrow and from progenitors in the embryonic yolk sac and fetal liver during early development.

Macrophage Events in Inflammation

Q. Mention the role of macrophages in chronic inflammation.

- **Monocytes** also emigrate into extravascular tissues early in acute inflammation, and **within 48 hours**, they are the **predominant cell type**.
- **On reaching extravascular tissue**, the monocyte is **transformed into** a larger phagocytic cell known as **tissue macrophage**.

Macrophage Activation

Tissue macrophages are activated by **two major pathways**:

- **Classical macrophage activation:**
 - **Mediators of activation:** It is brought out mainly by
 - ◆ Microbial products : For example, endotoxin
 - ◆ T cell-derived signals: Mainly cytokines (For example, IFN- γ)
 - ◆ Foreign substances: e.g. crystals and particulate matter
 - **Products of activated macrophages**
 - ◆ Lysosomal enzymes
 - ◆ Nitric oxide
 - ◆ Reactive oxygen species (ROS)
 - **Function:** Phagocytosis and killing/elimination of ingested microbes.
- **Alternate macrophage activation:**
 - **Mediators of activation:** It is brought out mainly by **cytokines** IL-4 and IL-13 produced by T-cells and other cells.
 - **Function:** Initiation of the tissue repair, (they are not bactericidal).

Functions of Macrophages in Inflammation

- **Phagocytosis:** Ingestion and elimination of microbes and necrotic tissue.
- **Initiation of the tissue repair.**

- **Secretion of mediators of inflammation:** These include cytokines (TNF, IL-1, chemokines, etc.) and arachidonic acid metabolites.
- **Display signal to T-cells and respond signals from T-cells:** This is responsible for the feedback loop for defense against many microbes by cell-mediated immune response.

Main cytokines involved in chronic inflammation:

(1) IL-12 (2) INF- γ (3) IL-17.

Lymphocytes

- **B and T-lymphocyte:** They are found in both antibody-mediated and cell-mediated immune reactions.
- **B lymphocytes:** They may develop into plasma cells and produce antibodies either against foreign or self-antigens in the inflammatory site.
- **T lymphocytes:** Important being **CD4⁺ helper T cells** which has **3 subtypes** namely:

Q. Write short note on T helper cell.

- **T_H1:** Produce INF- γ and activates macrophage in the classical pathway.
- **T_H2:** Produce IL-4, IL-5 and IL-13 which recruit and activate eosinophils and activate macrophages through alternate pathway. Involved in defense against helminthic infestation and allergic reaction.
- **T_H17:** Produce IL-17 and other cytokines which recruit neutrophils and monocytes.

CD4⁺ helper T cells are of 3 types: (1) T_H1 (2) T_H2 (3) T_H17.

Chronic inflammation: Infiltration by lymphocytes, macrophages and plasma cells, often with significant fibrosis. In chronic endometritis, there are plasma cells.

Viral infections: Lymphocytes are first cells to arrive at the site of inflammation.

Other Cells

- **Plasma cells (refer Chapter 2):** They are derived from activated B lymphocytes and produce antibodies either against foreign or self-antigens.
- **Eosinophils (refer Chapter 2):** They are seen in immune reactions mediated by IgE and in parasitic infections. A **chemokine**, which **attracts eosinophil recruitment is eotaxin**. Eosinophils granules contain **major basic protein** which is toxic to parasites and also destroy the epithelial cells.
- **Mast cells:** They are distributed in connective tissues and participate in both acute and chronic inflammatory

reactions. They are seen in allergic reactions to foods, insect venom, or drugs.

TYPES OF CHRONIC INFLAMMATION

It can be divided into (1) chronic-non-specific inflammation and (2) granulomatous inflammation.

Eosinophils are observed in:

- Hypersensitivity reactions
- Parasitic infestations.

Granulomatous Inflammation

Q. Define and classify granuloma.

Definition: A **granuloma** is defined as a **distinctive type of chronic inflammation** characterized by **microscopic aggregation of activated macrophages** (that are transformed into epithelium-like/epithelioid cells) with scattered lymphocytes. Older granulomas in addition show rim of fibroblasts and connective tissue as the outermost layer. Structurally, granuloma consists of:

Q. Write short note on epithelioid cell.

- **Epithelioid cells:** These are **modified macrophages** which resemble epithelial cells.
 - They have a **pale pink granular cytoplasm** with indistinct cell borders, often appearing to merge into one another.
 - The **nucleus is oval or elongate**, and may show folding of the nuclear membrane. The nucleus is less dense than that of a lymphocyte.
- **Giant cells:** **Epithelioid cells** frequently **fuse to form giant cells** and are found in the periphery or sometimes in the center of granulomas. These giant cells may attain diameters of 40–50 μm and have many small nuclei. Nuclei may be as many as 20 or more which are and may be arranged either peripherally (Langhans-type giant cell) or haphazardly (foreign body-type giant cell).
- **Lymphocytes:** As a cell-mediated immune reaction to antigen, lymphocytes form an integral part of granulomatous inflammation. Some types may be accompanied by plasma cells.
- **Necrosis:** Sometimes **granulomas are associated with central necrosis** (e.g. tuberculosis). However, the granulomas in Crohn disease, sarcoidosis, and foreign body reactions does not have necrotic centers and are called as *noncaseating granulomas*.
- **Fibrosis:** Granulomas may heal by producing extensive fibrosis.

Types of Granulomas

Depending on the pathogenesis there are of two types:

Foreign Body Granulomas

- It develops against relatively inert foreign bodies which do not incite any specific inflammatory or immune response (absence of T-cell-mediated immune responses).
- The foreign body which elicit granuloma include suture materials, talc (associated with intravenous drug abuse), or other fibers that are large enough to be phagocytosis by a macrophage. Epithelioid cells and giant cells are apposed to the surface of these foreign bodies.
- The foreign material can usually be found in the center of the granuloma, particularly if seen with polarized light (appears refractile).

Immune Granulomas

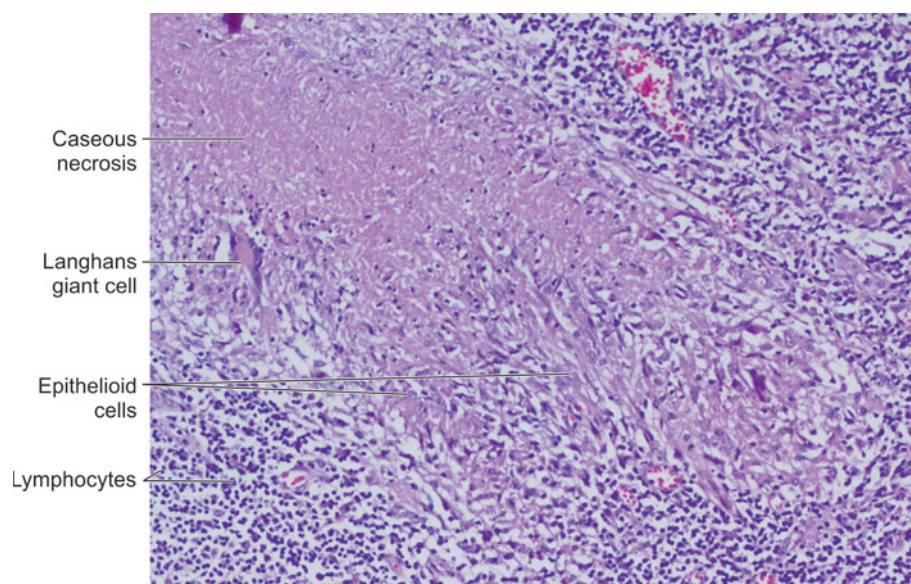
- These are caused by agents/microbes which are capable of inducing a persistent T-cell-mediated immune response.
- Immune granulomas usually develop when the inciting agent is difficult to eradicate, such as a persistent microbe (e.g. *Mycobacterium tuberculosis*) or a self-antigen. In these granulomas, macrophages activate T cells to produce cytokines, such as IL-2. This in turn activates other T cells, perpetuating the response, and IFN- γ , which activates the macrophages.
- Granuloma in tuberculosis (Fig. 4.1) is referred to as a tubercle and usually shows central caseous necrosis (due to a combination of hypoxia and free radical-mediated injury) and is rare in other granulomatous diseases. Sometimes, it may be necessary to perform additional tests/investigations to identify the etiologic agent.
 - Special stains, e.g. acid-fast stains for tubercle bacilli
 - Culture methods, e.g. in tuberculosis and fungal diseases
 - Molecular techniques (e.g. the polymerase chain reaction in tuberculosis)
 - Serologic studies (e.g. in syphilis).

Examples of granulomatous inflammation are listed in Table 4.1.

Granulomatous inflammation: Distinctive pattern of chronic inflammation. It is produced by few infectious as well as noninfectious conditions and involves immune reactions.

Epithelioid cells: Modified macrophages and morphologically resemble epithelial cells.

Epithelioid cells: Macrophages activated by INF- γ secreted by CD4⁺ T-cells.



Caseous necrosis;
 • Tuberculosis
 • Histoplasmosis.

Fig. 4.1: Tuberculous granuloma showing an area of caseous necrosis surrounded by epithelioid cells, Langhans-type giant cell, and lymphocytes

TABLE 4.1: Examples of granulomatous inflammation

Disease	Cause	Tissue reaction
Tuberculosis	<i>Mycobacterium tuberculosis</i>	Caseating granuloma (tubercle): central necrosis with amorphous granular debris surrounded by epithelioid cells, rimmed by lymphocytes, histiocytes and fibroblasts. Occasional Langhans giant cells, presence of acid-fast bacilli
Leprosy	<i>Mycobacterium leprae</i>	Acid-fast bacilli in macrophages; noncaseating granulomas
Syphilis	<i>Treponema pallidum</i>	Gumma: microscopic to grossly visible lesion. Consists of histiocytes; plasma cell infiltrate; central necrotic cells without loss of cellular outline (coagulative necrosis)
Cat-scratch disease	Gram-negative <i>Bacillus</i>	Rounded or stellate granuloma containing central granular debris and neutrophils; giant cells rare
Sarcoidosis	Unknown etiology	Noncaseating granulomas with plenty of activated macrophages
Crohn disease (inflammatory bowel disease)	Immune reaction against intestinal bacteria, self-antigens	Dense chronic inflammatory infiltrate with occasional noncaseating granulomas in the wall of the intestine

GIANT CELL

Q. Write short note on various types of giant cells and the conditions associated with it.

Definition: Cell with more than one nucleus is called as giant cell or multinucleated cell.

Types of giant cells (Fig. 4.2): Various types of giant cells and its associated conditions are mentioned in Box 4.1.

GRANULOMATOUS DISEASES

Mycobacterium is bacteria, which appear as slender aerobic rods that grow in straight or branching chains. Mycobacteria have a waxy cell wall composed of mycolic acid, which is

responsible for their **acid fast nature**. Mycobacteria are weakly Gram-positive.

Acid fast means that mycobacteria retain stains even on treatment with a mixture of acid and alcohol.

LEPROSY

Leprosy (Hansen disease-after the discovery of the causative organism by Hansen), is a **chronic, granulomatous**, slowly progressive, destructive infection caused by *Mycobacterium leprae*.

Sites of involvement: Mainly involves the **peripheral nerves, skin and mucous membranes** (nasal) and results in disabling deformities.

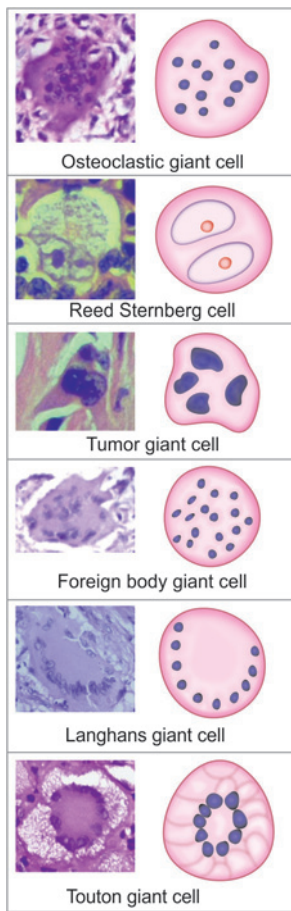


Fig. 4.2: Various types of giant cells

BOX 4.1: Types of giant cells

Physiological

Osteoclast

Syncytiotrophoblast

Megakaryocyte

Pathological

Damaged muscle fibers

- Regenerating sarcolemmal cells in damaged skeletal muscles

Tumor giant cells: They have hyperchromatic nuclei of varying size and shape

- Giant cell tumors: Bone (osteoclastoma)
- Reed Sternberg cells: Hodgkin lymphoma
- Giant cell variants of many malignant tumors, e.g. carcinoma of lung

Giant cells resulting from fusion of cells

- Viral infection
 - Epithelial giant cells, e.g. herpes virus infection
 - Connective tissue, e.g. Warthin-Finkeldey giant cells in measles

Fused macrophages

- Foreign body giant cells: These have multiple uniform nuclei scattered throughout the cytoplasm.
 - Reaction to exogenous insoluble material: For example, suture material, talc, etc.
 - Reaction to insoluble endogenous material: For example, keratin (dermoid cyst of ovary, epidermal cyst), cholesterol, urate crystals (in gout)
- Touton giant cells: These cells have vacuolated cytoplasm due to lipid, e.g. in xanthoma
- Reaction to certain organisms: For example, tuberculosis (Langhans giant cells in which nuclei are arranged in a horseshoe pattern), fungal infections, syphilis
- Fusion of cardiac histiocytes: Aschoff giant cells in rheumatic heart disease.

Leprosy is one of the oldest human diseases and lepers were isolated from the community in the olden days.

Mycobacterium Leprae

- Slender, **weakly acid-fast** intracellular bacillus. It closely resembles *Mycobacterium tuberculosis* but is less acid-fast.
- **Proliferates at low temperature** of the human skin.
- **Cannot be cultured** on artificial media or in cell culture.
- **Experimental animals:** Lepra bacilli grow at sites where the temperature is below that of the internal organs. Examples: **Foot pads of mice**, ear lobes of hamsters, rats, and other rodents.
- **Experimentally transmitted to nine banded armadillos** (they have low body temperature ranging from 32–34°C).
- **Antigen in lepra bacilli:** The bacterial cell wall contains mainly 2 antigens namely *M. leprae*-specific phenolic glycolipid (PGL-1) and lipoarabinomannan (LAM).

Mode of transmission: It has comparatively low communicability.

M. leprae: Grows best in cooler tissues: (1) Skin, (2) Peripheral nerves, (3) Anterior chamber of eye, (4) Upper respiratory tract and (5) Testis.

1. **Inoculation/inhalation:** Likely to be transmitted from person to person through aerosols from asymptomatic lesions in the upper respiratory tract. Inhaled *M. leprae*, is taken up by alveolar macrophages and disseminates through the blood, but replicates only in relatively cool tissues of the skin and extremities.
2. **Intimate contact:** For many years with untreated leprosy patients. They shed many bacilli from damaged skin, nasal secretions, mucous membrane of mouth and hair follicles.

Source of infection: *M. leprae* is present in nasal secretions or ulcerated lesions of patients suffering from leprosy.

Mycobacterium leprae: Only bacterium that invades peripheral nerves.

Incubation period: Generally 5–7 years.

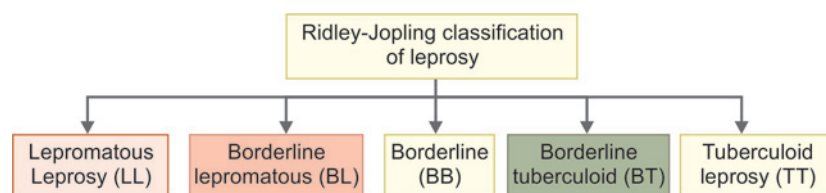


Fig. 4.3: Ridley-Jopling classification of leprosy

Classification of leprosy:

1. Ridley and Jopling classification
2. WHO classification.

Classification

Q. Classify leprosy.

A. Ridley and Jopling (1966) classification: It depends on the clinicopathological spectrum of the disease, which is **determined by the immune resistance of the host** (Fig. 4.3). They are classified into five groups with two extremes or polar forms, namely tuberculoid and lepromatous types.

1. **Tuberculoid leprosy (TT):** It is the polar form that has maximal immune response.
2. **Borderline tuberculoid (BT):** In this type, the immune response falls between BB and TT.
3. **Borderline leprosy (BB):** It exactly falls between two polar forms of leprosy.
4. **Borderline lepromatous (BL):** It has the immune response that falls between BB and LL.
5. **Lepromatous leprosy (LL):** It is the other polar form with least immune response.

Variants of Leprosy

- **Indeterminate leprosy:** It is an initial nonspecific stage of any type of leprosy.
- **Pure neural leprosy** in which neurologic involvement is the main feature. The skin lesions of leprosy are not seen.
- **Histoid leprosy:** It is a variant of lepromatous leprosy in which the skin lesions grossly resemble nodules of dermatofibroma and microscopically shows numerous lepra bacilli.

B. WHO classification:

Leprosy WHO classification: Paucibacillary and multibacillary.

- **Paucibacillary:** All cases of tuberculoid leprosy and some cases of borderline type.
- **Multibacillary:** All cases of lepromatous leprosy and some cases of borderline type.

Pathogenesis

- *Mycobacterium leprae* does not secrete any toxins, and its virulence depends on properties of its cell wall (similar

to that of *M. tuberculosis*) and immunization with BCG may provide some protection against *M. leprae* infection.

Tuberculoid leprosy has a strong T_H1 response compared to weak T_H1 response in lepromatous leprosy.

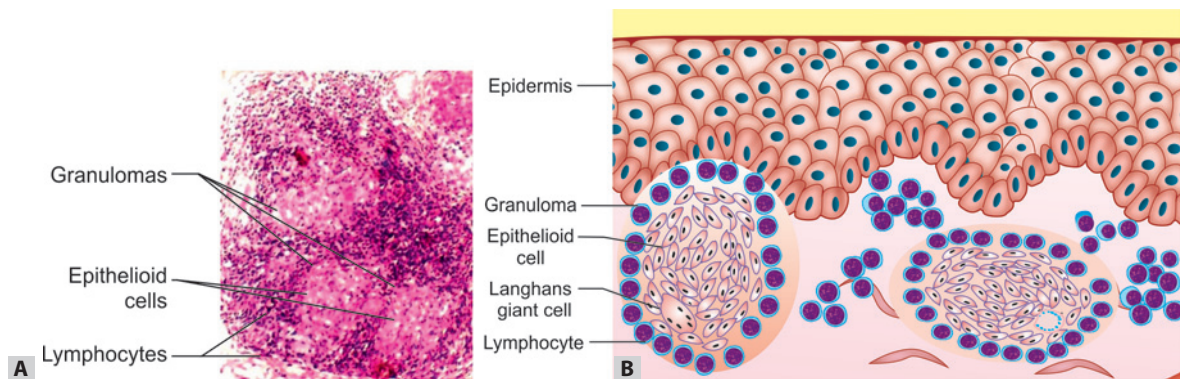
- Cell-mediated immunity is reflected by delayed-type hypersensitivity reactions to dermal injections of a bacterial extract called **lepromin**.
- The **T-helper (T_H1) lymphocyte response** to *M. leprae*, determines whether an individual develop tuberculoid or lepromatous type of leprosy.
 - **Tuberculoid leprosy** patients have a T_H1 response which secretes IL-2 and IFN- γ . The later (IFN- γ) is essential for an effective host macrophage response.
 - **Lepromatous leprosy** patients have a weak T_H1 response and, in some a relative increase in the T_H2 response → results in a poor cell-mediated immunity → proliferation of lepra bacilli. Sometimes antibodies may be produced against *M. leprae* antigens, but they are usually not protective. These can form immune complexes with free antigens and lead to erythema nodosum, vasculitis, and glomerulonephritis.

MORPHOLOGY

Q. Write short note on morphology of tuberculoid leprosy.

Two extremes or polar forms of the diseases are the tuberculoid and lepromatous types.

- **Tuberculoid leprosy:** It is the **less severe form** of leprosy. It is **very slow in its course** and most patients die with leprosy.
 - **Lesion in skin:**
 - ♦ **Number of lesions:** Single or very few lesions.
 - ♦ **Site:** Usually on the **face, extremities, or trunk**
 - ♦ **Type:** **Localized, well-demarcated, red or hypopigmented, dry, elevated, skin patches** having raised outer edges and depressed pale centers (central healing). As they progress they develop irregular shapes with induration.
 - **Nerve involvement:**
 - ♦ **Dominating feature** in tuberculoid leprosy.
 - ♦ Nerves are surrounded by granulomatous inflammatory reactions and, may destroy small (e.g. the peripheral twigs) nerves.
 - ♦ Nerve involvement → causes **loss of sensation** in the skin → atrophy of skin and muscle. These affected parts are liable to trauma, and lead to the development of chronic skin ulcers.



Figs 4.4A and B: Microscopy of tuberculoid leprosy with circumscribed non-caseating granulomas. (A) Photomicrograph; (B) Diagrammatic

- ◆ **Consequences:** It may lead to **contractures, paralyses, and autoamputation** of fingers or toes. Involvement of facial nerve can lead to paralysis of the eyelids, with keratitis and corneal ulcerations.
- **Microscopy** (Fig. 4.4):
 - ◆ **Granuloma:** These are well-formed, circumscribed and **non-caseating** (no caseation). Seen in all involved sites and in the dermis of skin. Termed tuberculoid leprosy because the granulomas resemble those found in tuberculosis. Granulomas are composed of epithelioid cells (modified macrophages), Langhans giant cells, and lymphocytes.
 - ◆ **Absence of Grenz zone:** Granulomas in the dermis extend to the basal layer of the epidermis (without a clear/Grenz zone).
 - ◆ **Fite-Faraco** (modified Z-N stain for demonstration of lepra bacillus) stain generally **does not show lepra bacillus**, hence the name “paucibacillary” leprosy.
 - ◆ **Perineural** (surrounding nerve fibers) **inflammation:** By lymphocytes.
 - ◆ **Strong T-cell immunity:** It is responsible for granulomas formation, without lepra bacilli.

Tuberculoid leprosy:

1. Good immune response
2. Lepromin test positive
3. Noncaseating granuloma in the skin
4. Nerve involvement.

- **Lepromatous leprosy:** It is the **more severe form** and is also called **anergic leprosy**, because of the unresponsiveness (anergy) of the host immune system.

Q. Write short note on morphology of lepromatous leprosy.

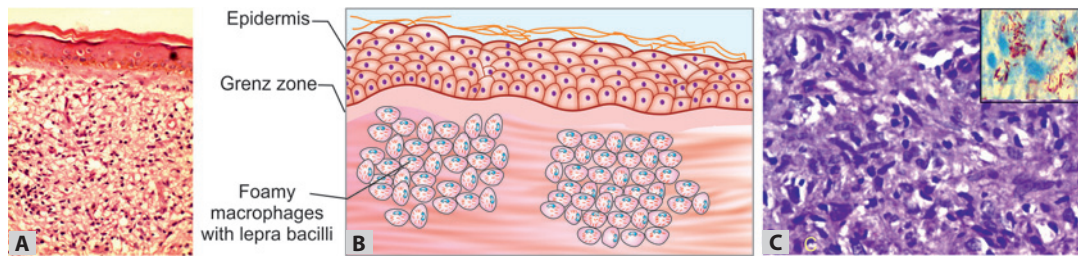
Sites involved:

- **Lesion in skin:**
 - ◆ **Thickening of skin and multiple, symmetric, macular, papular, or nodular lesions.** The nodular skin lesions may ulcerate. Most skin lesions are **hypoesthetic or anesthetic**.



Fig. 4.5: Leonine facies of lepromatous leprosy

- ◆ More severe involvement of the **cooler areas of skin** (e.g. earlobes, wrists, elbows, and knees, and feet), than warmer areas (e.g. axilla and groin).
- ◆ With progression, the nodular lesions in the face and earlobes may coalesce to produce a lion like appearance known as **leonine facies** (Fig. 4.5). This may be accompanied by loss of eyebrows and eyelashes.
- **Peripheral nerves:**
 - ◆ Particularly the **ulnar and peroneal nerves** are symmetrically invaded with mycobacteria.
 - ◆ **Loss of sensation and trophic changes** in the hands and feet may follow the damage to the nerves.
- **Testes:** Usually, severely involved, leading to destruction of the seminiferous tubules → sterility.
- **Other sites:**
 - ◆ Anterior chamber of the eye: **Blindness**.
 - ◆ Upper airways: Chronic nasal discharge and voice change.



Figs 4.6A to C: Microscopic appearance of lepromatous leprosy. (A) Photomicrograph. (B) Diagrammatic. The epidermis is thinned and the dermis shows dense collections of lepra cells. The epidermis is separated from the collections of lepra cells by an uninvolved Grenz zone; (C) Photomicrograph. High power view showing foamy macrophages. Inset of C shows acid-fast lepra bacilli within macrophages (Fite-Faraco stain).

– **Microscopy of skin lesion** (Fig. 4.6):

- ♦ **Flattened epidermis:** Epidermis is thinned and flattened (loss of rete ridges) over the nodules.
- ♦ **Grenz (clear) zone:** It is a characteristic **narrow, uninvolved dermis** (normal collagen) which separates the epidermis from nodular accumulations of macrophages.

Q. Write short note on lepra cell.

- ♦ **Lepra cells:** The nodular lesions contain large aggregates of lipid-laden foamy macrophages (lepra cells, Virchow cells), filled with aggregates ("globi") of acid-fast lepra bacilli (*M. leprae*).
- ♦ **Fite-Faraco (acid-fast) stain:** It shows **numerous lepra bacilli** ("red snappers") within the foamy macrophages. They may be arranged in a parallel fashion like **cigarettes in a pack**.
- ♦ Due to the presence of numerous bacteria, lepromatous leprosy is also referred to as "**multibacillary**".

In advanced cases, *M. leprae* may be present in sputum and blood.

Fite-Faraco stain: Modified Z-N stain used for demonstration of lepra bacilli in tissue.

Lepromatous leprosy:

- Grenz zone is a narrow, uninvolved dermis that separates epidermis from macrophages
- Lepra cells are large lipid-laden macrophages filled with *M leprae*.

Virchow (lepra/foam) cells are diagnostic of LL.

Individual with intermediate forms of disease, called **borderline leprosy**.

3. **Borderline leprosy:**

- **Borderline tuberculoid (BT)** shows epithelioid cells and numerous lymphocytes with a narrow clear subepidermal zone. Lepra bacilli are few and found in nerves.
- **Borderline lepromatous (BL)** shows predominantly of histiocytes, few epithelioid cells and lymphocytes. Numerous lepra bacilli are found.

- **Mid-borderline (BB)** or dimorphic form shows sheets of epithelioid cells without any giant cells. Few lymphocytes are found in the perineurium. Lepra bacilli are seen mostly in nerves.

4. **Indeterminate leprosy:** **Microscopically**, features are non-specific and few findings help in suspecting leprosy. These include: (1) local infiltration of lymphocytes or mononuclear cells surrounding the skin adnexa (e.g. hair follicles and sweat glands) or around blood vessels, (2) involvement of nerve involvement (if seen strongly favors the diagnosis) and (3) finding of lepra bacilli (which confirms the diagnosis).

Lepromin Test

It is **not a diagnostic test** for leprosy. It is **used for classifying** the leprosy based on the immune response.

- **Procedure:** An antigen extract of *M. leprae* called lepromin is intradermally injected.

Q Write short note on Mitsuda reaction.

- **Reaction:**
 - An **early positive reaction** appears as an indurated area in 24–48 hours is called **Fernandez reaction**.
 - A **delayed granulomatous reaction** appearing after 3–4 weeks is known as **Mitsuda reaction**.
- **Interpretation:**
 - **Lepromatous leprosy**—shows **negative lepromin test** due to suppression of cell-mediated immunity.
 - **Tuberculoid leprosy**—show **positive lepromin test** because of delayed hypersensitivity reaction.

Lepromatous leprosy:

1. Leonine facies
2. Low resistance
3. Thinned epidermis
4. Grenz zone
5. Lepra cells filled with acid-fast bacilli
6. Lepromin test negative.

Uses of lepromin test:

1. Classification of leprosy
2. Evaluation of cell-mediated immunity status in patient
3. Know the prognosis.

Reactions in Leprosy

The immunity in leprosy may change spontaneously or following treatment.

• Type I reaction:

- **Borderline leprosy** is the **most unstable form of leprosy** where **immune status may shift up or down**. These are called as type I reaction, which may be of two types:
 - ◆ Upgrading reactions: If immunity improves, the disease may shift towards tuberculoid leprosy.
 - ◆ Downgrading reaction: If the immunity decreases, the disease moves towards lepromatous leprosy.

• Type II reaction or erythema nodosum leprosum:

- It occurs in **mostly in lepromatous leprosy**, particularly when on treatment.
- **Clinical features:** (1) Tender red plaque or nodules and (2) fever, malaise and arthralgia.
- **Microscopy:**
 - ◆ Necrotizing vasculitis
 - ◆ Lepra bacilli in the foamy macrophages.

Differences between lepromatous and tuberculoid leprosy are presented in Table 4.2.

Diagnosis of Leprosy

1. Clinical examination:

- **Sensory testing**
- Examination of peripheral nerve

TABLE 4.2: Differences between lepromatous and tuberculoid leprosy

Q. List the differences between lepromatous and tuberculoid leprosy.

Characteristics	Lepromatous leprosy	Tuberculoid leprosy
Clinical features		
Skin lesions	Symmetrical, multiple, ill-defined, macular, nodular	Asymmetrical, hypopigmented, well-defined macular
Disfigurement	Leonine facies, loss of eyebrows, pendulous earlobes, claw-hands, saddle nose	Minimal disfigurement
Nerve involvement	Seen, but with less severe sensory loss than tuberculoid	Common with sensory disturbances
Microscopy of skin lesions		
Type of lesion	Nodular or diffuse collections of Lepra cells within dermis	Noncaseating granulomas composed of epithelioid cells and giant cells
Grenz/clear zone between inflammatory cells and epidermis	Present	Absent
Lepra bacilli	Plenty within the lepra cells as globular masses (globi)	Rare if any
Bacillary index	4 or 5	0
Other features		
Immunity	Suppressed-low resistance	Good immunity-high resistance
Lepromin test	Negative	Positive

Diagnosis of Leprosy

- Staining of smears or skin biopsy
 - Acid fast (Ziehl Neelsen) stain
 - Fite–Faraco stain
- Molecular method—PCR

Morphological index (MI):

- Measure of number of acid-fast bacilli (AFB) in skin scrapings that stain uniformly bright.
- Correlates with viability of AFB.

Bacteriological index (BI): Quantifies *M. leprae* in tissue or smears. It scored from 1+ to 6+ (range from 1 to 10 bacilli per 100 fields to > 1000 per field) as multibacillary leprosy whereas BI of 0 + is termed paucibacillary.

2. **Demonstration of acid-fast bacilli:**

- Skin smears prepared by **slit and scrape method**
- *Mycobacterium leprae* can be demonstrated in tissue sections, in split skin smears by splitting the skin, s, and in nasal smears by the following techniques:
 - Acid-fast (Ziehl-Neelsen) staining.
 - Fite-Faraco staining procedure is a modification of ZN procedure and is considered better for more adequate staining of tissue sections (Fig. 4.6C).
 - Gomori methenamine silver (GMS) staining can also be employed.
- **Nasal swabs stained by Ziehl-Neelsen (ZN) method.** The staining procedure is similar to that procedure employed for *M. tuberculosis* but can be decolorized by lower concentration (5%) of sulfuric acid (less acid-fast).

3. **Skin biopsy:** Fite-Faraco staining procedure is a modified ZN procedure and is better for tissue sections.4. **Nerve biopsy**5. **Molecular method:** Polymerase chain reaction (PCR).

- Cannot be grown in artificial media.
- **Staining:** It does not stain with ordinary bacterial stains and is too slender to be seen in Gram stain. It can be visualized by **silver stains, dark-field examination, and immunofluorescence techniques.**
- **Source of infection:** An open lesion of **primary or secondary syphilis**. Lesions in the mucous membranes or skin of the genital organs, rectum, mouth, fingers, or nipples.
- **Mode of transmission:**
 - **Sexual contact:** It is the usual mode of spread.
 - **Transplacental transmission:** From mother with active disease to the fetus (during pregnancy) → congenital syphilis.
 - **Blood transfusion.**
 - **Direct contact:** With the open lesion is rare mode of transmission.

SYPHILIS

IgM antibodies to PGL-1 antigen: Found in 95% of patients of lepromatous leprosy and in 60% of tuberculoid leprosy.

Syphilis: Caused by spirochete *Treponema pallidum*.

Introduction: Spirochetes are Gram-negative, slender corkscrew-shaped bacteria covered in a membrane called an outer sheath, which may mask its antigens from the host immune response.

Syphilis (lues) is a **chronic, sexually transmitted disease caused by spirochete *Treponema pallidum*.**

Etiology

Treponema pallidum (Fig. 4.7):

- It is a **thin, delicate, corkscrew-shaped spirochete**, measures about 10 µm long with tapering ends and has about 10 regular spirals.
- Actively motile, showing rotation round the long axis, backward and forward motion.

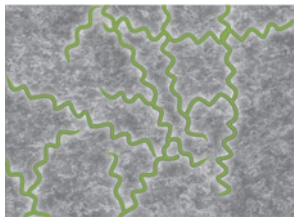


Fig. 4.7: Diagrammatic appearance of *Treponema pallidum* under Dark-field examination

Basic Microscopic Lesion

Irrespective of stage, the basic microscopic lesion of syphilis consists of:

- **Mononuclear inflammatory infiltrate:** Predominantly of **plasma cells and lymphocytes.**
- **Obliterative endarteritis:** It is a characteristic obstructive vascular lesion in which mononuclear infiltrates surround small arteries and arterioles (**periarteritis**).

Stages of Syphilis (Fig. 4.8)

Treponema pallidum passes from the site of inoculation to regional lymph nodes and enters to the systemic circulation, and disseminate throughout the body. Syphilis can be **(1) congenital or (2) acquired**. The course of acquired syphilis is divided into three stages:

- Primary syphilis
- Secondary syphilis
- Tertiary syphilis.

Primary Syphilis

Develops about **3 weeks after contact** with an infected individual and the lesion is primary chancre.

Primary Chancre

Q. Write short note on primary chancre.

It is the classical lesion of primary syphilis.

- **Sites:** **Penis or scrotum** in men and **cervix, vulva and vaginal wall** in women. It may also be seen in the anus or mouth.

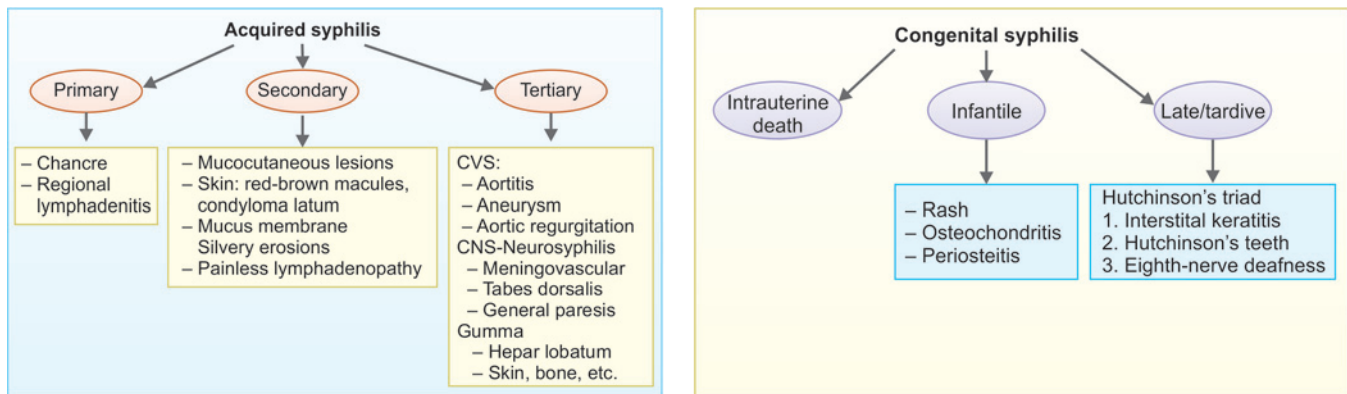


Fig. 4.8: Various manifestations of syphilis

Abbreviation: CVS, cardiovascular system; CNS, central nervous system

- **Gross features:** It is single, firm, **nontender (painless)**, slightly raised, **red papule** (chancre) up to several centimeters in diameter. It erodes to create a clean-based shallow ulcer. Because of the induration surrounding the ulcer, it is designated as **hard chancre**.
- **Demonstration of treponema:** Plenty of treponemes can be demonstrated in the chancre by (1) silver stains (e.g. Warthin-Starry stain) or (2) immunofluorescence techniques or (3) Dark-field examination.
- **Microscopy:**
 - **Mononuclear infiltration:** Consisting of **plasma cells**, with scattered macrophages and lymphocytes. These cells are also seen surrounding the blood vessels (periarteritis).
 - **Blood vessels with endarteritis:** It is characterized by endothelial cell proliferation which progresses to intimal fibrosis.

Primary syphilis: Chancre is the painless lesion seen in the external genitalia with regional lymphadenitis.

Regional Lymphadenitis

It is due to nonspecific acute or chronic inflammation.

- Treponemes may spread throughout the body by blood and lymphatics even before the appearance of the chancre.
- **Symptoms:** Usually, painless and often unnoticed.
- **Fate:** It heals in 3–6 weeks with or without therapy.

Secondary Syphilis

Secondary syphilis:

1. Mucocutaneous lesions
2. Generalized lymphadenopathy.

Q. Write short note on secondary syphilis.

It develops 2–10 weeks after the primary chancre in approximately 75% of untreated patients. Its manifestations are due to systemic spread and proliferation of the spirochetes within the skin and mucocutaneous tissues.

LESIONS OF SECONDARY SYPHILIS

Q. Write short note on anogenital syphilis.

Mucocutaneous Lesions

These are painless, superficial lesions and contain spirochetes and are infectious.

- **Skin lesions:**
 - **Skin rashes:** Consist of **discrete red-brown macules** less than 5 mm in diameter, but it may be scaly/pustular/annular. They are more frequent on the **palms** of the hands, or **soles** of the feet.
 - **Condylomata lata:** These are **broad-based, elevated plaques** with numerous spirochetes. They are seen in moist areas of the skin, such as the **anogenital region (perineum, vulva, and scrotum)**, inner thighs, and axillae.
 - **Mucosal lesions:** Usually occurs in the mucous membranes of **oral cavity or vagina as silvery-gray superficial erosions**. These lesions contain numerous *T. pallidum* and are the **highly infectious**.
- Microscopy:** Similar to primary chancre, i.e. infiltration by plasma cells and endarteritis obliterans.

Painless Lymphadenopathy

Especially involves **epitrochlear nodes** and shows plenty of spirochetes.

Symptoms: Mild fever, malaise, and weight loss are common in secondary syphilis, which may last for several weeks. The lesions subside even without treatment.

Treponema pallidum: It can be identified in lesions of primary or secondary syphilis.

Tertiary Syphilis

Q. Write short note on tertiary syphilis.

Tertiary syphilis: Involves mainly CVS, CNS and focal lesions called gumma.

- After the lesions of secondary syphilis have subsided patients enters an asymptomatic latent phase of the disease.
- The latent period may last for 5 years or more (even decades), but spirochetes continue to multiply.
- This stage is rare if the patient gets adequate treatment, but can occur in about one-third of untreated patients.
- Focal ischemic necrosis due to obliterative endarteritis is responsible for many of the processes associated with tertiary syphilis.

Manifestations: Three main manifestations of tertiary syphilis are: **cardiovascular syphilis, neurosyphilis, and so-called benign tertiary syphilis**. These may occur alone or in combination.

Cardiovascular Syphilis

Most **frequently involves the aorta** and known as syphilitic aortitis.

- **Syphilitic aortitis:** Accounts for more than 80% of cases of tertiary disease, and affects the **proximal aorta**.
- **Saccular aneurysm and aortic valve insufficiency:**
 - **Occlusion of the vasa vasorum** due to endarteritis leads to **necrosis and scarring of the aortic media**, causing a loss of elasticity, strength and resilience.
 - Gradual weakening and slow progressive **dilation of the aortic root and arch**, causes **aortic valve insufficiency** and **aneurysms of the proximal aorta**. Syphilitic aneurysms are saccular and seen in the ascending aorta, which is unusual site for the more common atherosclerotic aneurysms.
 - On gross examination, the aortic intima appears rough and pitted (**tree-bark appearance**).
- **Myocardial ischemia:** Narrowing of the coronary artery ostia (at the origin from aorta) caused by subintimal scarring may lead to myocardial ischemia/infarction.

Cardiovascular syphilis: Involves proximal aorta and lead to saccular aneurysm of aorta and aortic valve incompetence. **Syphilis never causes aortic stenosis.**

Neurosyphilis

It may be asymptomatic or symptomatic.

- **Asymptomatic neurosyphilis:** It is detected by CSF examination, which shows pleocytosis (increased numbers of inflammatory cells), elevated protein levels, or decreased glucose. Antibodies can also be detected in the CSF, which is the most specific test for neurosyphilis.
- **Symptomatic disease:** Takes one of several forms
 - **Chronic meningovascular disease:** Chronic meningitis → involves base of the brain, cerebral convexities and spinal leptomeninges.
 - **Tabes dorsalis:** It is characterized by **demyelination of posterior column, dorsal root and dorsal root ganglia**.
 - **General paresis of insane:** Shows generalized brain parenchymal disease with **dementia**; hence called as general paresis of insane.

Neurosyphilis:

1. Chronic meningovascular disease
2. Tabes dorsalis
3. General paresis of insane.

Benign Tertiary Syphilis

It is characterized by the formation of nodular lesions called **gummas** in any organ or tissue. Gummas reflect development of delayed hypersensitivity to the spirochete. Gummas are very rare and may be found in patients with acquired immune deficiency syndrome (AIDS).

SYPHILITIC GUMMAS

Syphilitic gumma: Central area of coagulative necrosis surrounded by plump, palisading macrophages, fibroblasts and plenty of plasma cells.

Q. Write short note on gumma.

- May be single or multiple.
- **White-gray and rubbery.**
- Vary in size from microscopic lesions to large tumor-like masses.
- **Site:** They occur in most organs but mainly involve
 - **Skin, subcutaneous tissue** and the mucous membranes of the upper airway and mouth.
 - **Bone and joints:** It causes local pain, tenderness, swelling, and sometimes pathologic fractures.
 - In the **liver**, scarring due to gummas may cause a distinctive hepatic lesion known as **hepar lobatum**.
- **Microscopy:** Center of the gummas show **coagulative necrosis** → surrounded by plump, **palisading macrophages**, fibroblasts and plenty of **plasma cells**. Treponemes are scant in gummas.

Congenital Syphilis

Q. Write short note on congenital syphilis.

Transplacental Transmission

- *T. pallidum* can cross placenta and spread from infected **mother to the fetus** (during pregnancy).
- Transmission occurs, when **mother is suffering from primary or secondary syphilis** (when the spirochetes are abundant. Because of routine serologic testing for syphilis in done in all pregnancies) congenital syphilis is rare.

Manifestations: can be divided into:

1. **Intrauterine death and perinatal death.**
2. **Early (infantile) syphilis:** It occurs in the **first 2 years of life** and often manifested by **nasal discharge** and **congestion (snuffles)**.
 - A **desquamating or bullous eruption/rash** can lead to epidermal sloughing of the skin, mainly in the hands, feet, around the mouth and anus.
 - **Skeletal abnormalities:**
 - **Syphilitic osteochondritis:** Inflammation of bone and cartilage is more distinctive in the nose. Destruction of the vomer causes collapse of the nasal bridge → produces characteristic **saddle nose deformity**.
 - **Syphilitic periostitis:** It involves the tibia and causes excessive new bone formation on the anterior surfaces and leads to anterior bowing, or **saber shin**.

Q. Write short note on hepar lobatum.

- **Liver:** Diffuse fibrosis in the liver called as hepar lobatum.
 - **Lungs:** Diffuse interstitial fibrosis → lungs appear pale and airless (**pneumonia alba**).
3. **Late (tardive) syphilis:** Manifests **2 years after birth**, and about 50% of untreated children with neonatal syphilis will develop late manifestations.

Q. Write short note on components of Hutchinson's triad.

- **Manifestations:** Distinctive manifestation is **Hutchinson's triad** are:
 - **Interstitial keratitis.**
 - **Hutchinson's teeth:** They are like small screw-drivers or peg-shaped incisors, with notches in the enamel.
 - **Eighth-nerve deafness.**

Congenital syphilis: Caused by maternal transmission of *T. pallidum*.

Congenital syphilis: May lead to intrauterine death or infantile syphilis with widespread injury to skin, liver, bone and lungs.

Hutchinson's triad: (1) Interstitial keratitis (2) Hutchinson's teeth (3) Eighth-nerve deafness.

Laboratory Diagnosis

- **Immunofluorescence of exudate** from the chancre is important for diagnosis in primary syphilis.
- Microscopy and PCR are also useful.
- **Serological tests:**
 - **Nontreponemal antibody tests:** These tests measure antibody to cardiolipin, a phospholipid present in both host tissues and *T. pallidum*.

Q. Write short note on false-positive VDRL test.

- These antibodies are detected by the rapid plasma reagin and Venereal Disease Research Laboratory (VDRL) tests.
- **False-positive VDRL test:** Found in certain acute infections, collagen vascular diseases (e.g. systemic lupus erythematosus), drug addiction, pregnancy, hypergammaglobulinemia of any cause, and lepromatous leprosy.
- **Antitreponemal antibody tests:** These measure antibodies, which react with *T. pallidum*. These include:
 - **Fluorescent treponemal antibody absorption test (FTA)**
 - **Microhemagglutination assay for *T. pallidum* antibodies.**

Jarisch-Herxheimer reaction:

- Treatment of syphilitic patients having a high bacterial load, by antibiotics can cause a massive release of endotoxins, and cytokine that may manifest with high fever, rigors, hypotension, and leukopenia.
- This **syndrome** is called the Jarisch-Herxheimer reaction, which **can develop not only in syphilis but also in other spirochetal diseases**, such as Lyme disease.

TUBERCULOSIS

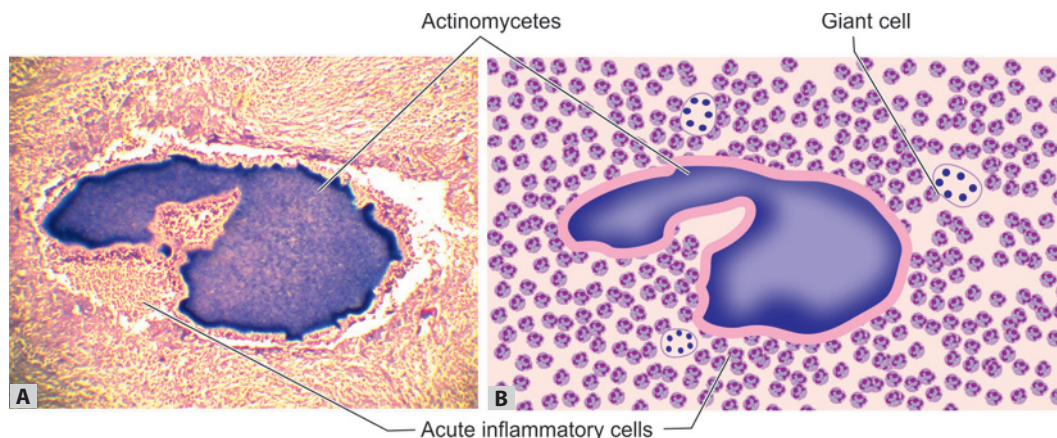
Refer Chapter 16.

OTHER INFECTIONS

Q. Write short note on actinomycosis.

Actinomycosis

- It is a chronic suppurative disease caused by anaerobic bacteria, *Actinomyces israelii*. It is not a fungus.
- The organisms are commensals in the oral cavity, gastrointestinal (GI) tract and vagina.



Figs 4.9A and B: Microscopy of actinomycosis showing central bacterial colony surrounded inflammatory cells. (A) Hematoxylin and eosin and (B) Diagrammatic

- **Mode of infection:** Infection is always endogenous in origin and not due to personal contact.
- **Break in mucocutaneous continuity, diminished immunity** due to some underlying disease favors the organism to invade, proliferate and disseminate.

MORPHOLOGY

Depending on the anatomic location of lesions, actinomycosis is divided into four types:

1. **Cervicofacial actinomycosis:**
 - It is the most common form (60%) and has best prognosis.
 - Infection gains through tonsils, carries teeth, periodontal diseases or trauma following extraction of tooth.
 - In the beginning, a firm swelling develops in the lower jaw (i.e. lumpy jaw). Later, the mass breaks down and forms abscess and sinuses. Typically, the sinus discharges yellow sulfur granules. The infection may spread into the adjacent soft tissues and may destroy the bone.
2. **Thoracic actinomycosis:**
 - The infection of lung is as a result of aspiration of organism from the oral cavity or extension of infection from abdominal or hepatic lesions.
 - Initially, lung lesions resemble pneumonia but as the disease progresses it spreads to the whole lung, pleura, ribs and vertebrae.
3. **Abdominal actinomycosis:**
 - The common sites are appendix, cecum and liver.
 - The infection occurs as a result of swallowing of organism from oral cavity or as an extension from thoracic cavity.
4. **Pelvic actinomycosis:** It develops as a complication of intrauterine contraceptive devices (IUCDs).

Microscopy (Fig. 4.9)

Following features are seen irrespective of the location of actinomycosis:

- **Granulomatous reaction with central suppuration:** There is formation of abscesses in the center of lesions and the periphery of the lesions shows chronic inflammatory cells, giant cells and fibroblasts.
- **The central abscess** contains bacterial colony (Sulfur granule) characterized by radiating filaments (was called as ray fungus) surrounded by hyaline, eosinophilic, club-like ends which represent immunoglobulins.
- **Special stains for bacteria:** The organisms are Gram positive filaments and nonacid-fast. They stain positively with Gomori's methenamine silver (GMS) stain.

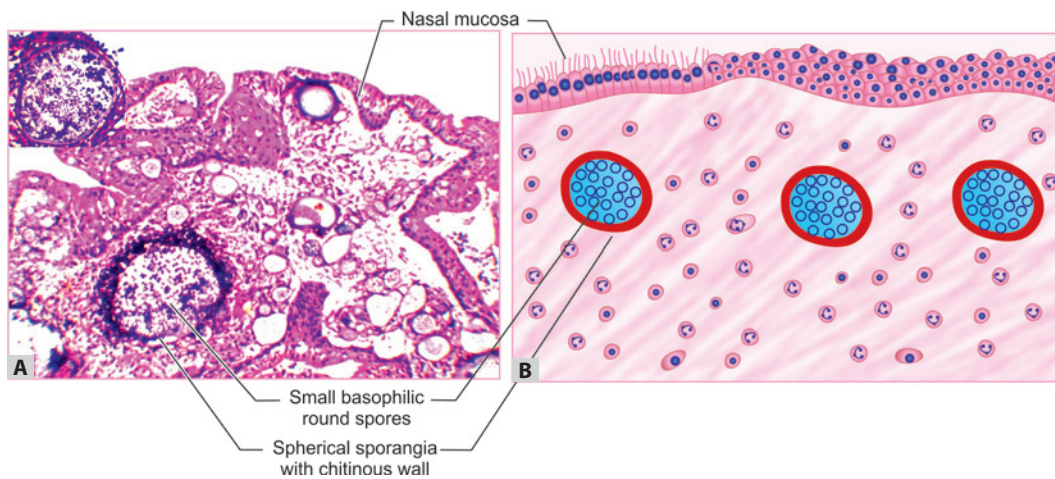
Rhinosporeidiosis

Q. Write short note on Rhinosporeidiosis.

Rhinosporeidiosis is an inflammatory disease caused by *Rhinosporeidium seeberi*. Usually, it occurs in nasopharynx as polyp but may also be observed in larynx and conjunctiva. It is endemic in India and Sri Lanka and sporadic in other parts of the world.

Microscopy (Fig. 4.10)

- Structure of nasal mucosa.
- Many spherical cysts called as sporangia measuring up to 200 nm in diameter having thick-walled (chitinous wall) are seen. Each of these cysts (i.e. sporangium) contains numerous small basophilic round spores of the size of erythrocytes. On rupture of a sporangium, the spores may be discharged into the submucosa or on to the surface of the mucosa.
- Chronic inflammatory (plasma cells, lymphocytes, histiocytes, neutrophils) infiltrate in the intervening and subepithelial layer.



Figs 4.10A and B: Rhinosporidiosis of nasopharynx showing spherical sporangia (A) Hematoxylin and eosin (H & E) and B Diagrammatic

Molluscum Contagiosum

Q. Write short note on molluscum contagiosum.

Molluscum contagiosum is a common, self-limited, highly contagious viral disease of the skin caused by a double-stranded DNA poxvirus.

Mode of infection: Usually spread by **direct contact**. Common among children and young adults.

Lesions: Infection leads to multiple lesions on the skin and mucous membranes, with a predilection for face, trunk and anogenital areas. Individual lesions are small, firm, smooth, often pruritic, pink to skin-colored, **dome-shaped papules**, generally ranging in diameter from 2 mm to 4 mm. Fully developed lesions have a characteristic **central umbilication** and in a fully-developed lesion, small amount of cheesy (curd/paste-like) keratinous material can be expressed on pressing from the central umbilication. This material if smeared onto a glass slide and stained with Giemsa may shows diagnostic molluscum bodies.

Microscopy (Fig 4.11): The microscopic picture is characteristic.

- **Infected epithelial cells:** Typical lesion consists of a sharply circumscribed (delimited) lobulated, cup-shaped mass of proliferating infected epithelial cells of epidermis growing down into the dermis.
- **Molluscum body:** As the infected epithelial cells differentiate within the mass, their cytoplasm is gradually filled by viral inclusion. These inclusions

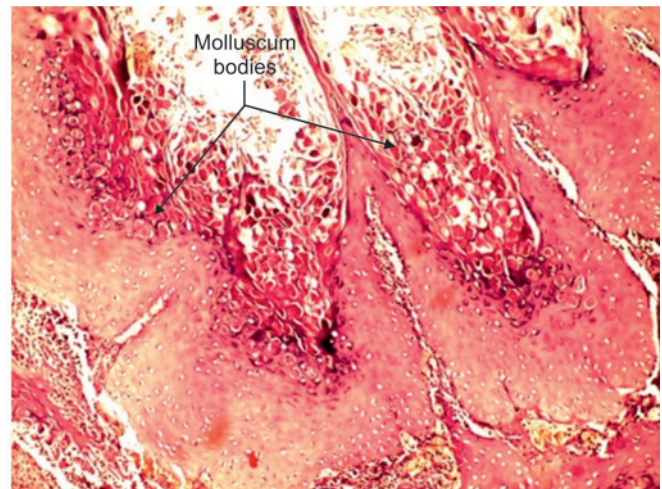


Fig. 4.11: Molluscum contagiosum. Epithelial cells of epidermis show ellipsoid cytoplasmic inclusions

enlarges the epithelial cells and displace the nucleus. The viral inclusions are diagnostically specific structure (which appear ellipsoid) and are termed as **molluscum body**. The viral inclusions are found in cells of the stratum granulosum and the stratum corneum. Under hematoxylin and eosin stain, these inclusions appear **faintly granular eosinophilic in the blue-purple stratum granulosum and pale blue in the red stratum corneum**. These molluscum bodies contain numerous viral particles. Most lesions spontaneously regress.

Hemodynamic Disorders, Thromboembolism and Shock

HYPEREMIA AND CONGESTION

Q. Write short note on hyperemia.

Hyperemia and congestion are characterized by **locally increased blood volume**.

Hyperemia

Definition: Hyperemia is an **active process** in which **arteriolar dilation** leads to increased **blood flow to a tissue/organ**.

Causes

- **Physiological:** Response to **increased functional demand** (e.g. heart and skeletal muscle during exercise).
- **Pathological:** Seen in **inflammation** and is responsible for the two cardinal signs of inflammation namely **heat** (calor) and **redness** (rubor/erythema).

Congestion

Q. Write short note on chronic passive congestion.

Definition: Congestion is a **passive process** resulting from **reduced venous outflow of blood** from a tissue/organ.

Types and Causes

1. **Systemic:** For example, congestive heart failure, congestion involves liver, spleen, and kidneys.
2. **Local:** For examples:
 - Congestion of leg veins due to deep venous thrombosis → edema of the lower extremity.

- Local congestion at various sites due to compression of veins, e.g. tight bandage, plasters, tumors, pregnancy, hernia, etc.

Onset

1. **Acute congestion:** It develops during shock, or sudden right-sided heart failure. It may occur in lung and liver.
2. **Chronic passive congestion:** It usually produces edema in the organ/tissue in which the venous outflow is reduced.
 - **Appearance:** Congested tissues have a **dark reddish-blue color (cyanosis)** due to stasis of RBCs and the accumulation of deoxygenated hemoglobin.

Hyperemia: Active process whereas congestion is a passive process. Both are characterized by locally increased blood volume.

Chronic Venous Congestion of Lung

Q. Write short note on CVC lung/brown induration of lung.

Causes

- **Mitral stenosis:** For example, rheumatic mitral stenosis.
- **Left-sided heart failure:** It develops secondary to coronary artery disease or hypertension.

Mechanism

- Chronic left ventricle failure → reduces the flow of blood out of the lungs → leads to chronic passive pulmonary congestion → **increases pressure in the alveolar capillaries** and they become excessively filled with blood.

Consequences

Q. Write short note on heart failure cells and the special stain used for its demonstration.

Four major consequences are:

- **Microhemorrhages:** The wall of alveolar capillaries may rupture → minute hemorrhages into the alveolar space → release RBCs → hemoglobin breakdown → **liberation of iron containing hemosiderin pigment** (brown color) → alveolar macrophages phagocytose hemosiderin. **Hemosiderin-laden macrophages** are known as **heart failure cells**.
- **Pulmonary edema:** It is due to forced movement of fluid from congested vessels into the alveolar spaces.
- **Fibrosis:** It develops due to increased fibrous tissue in the interstitium of lung.
- **Pulmonary hypertension:** It is due to transmission of pressure from the alveolar capillaries to the pulmonary arterial system.

Heart failure cells:

1. Hemosiderin-laden macrophages
2. Found in lung affected by CVC lung and not in the heart.

Heart failure cells: Hemosiderin pigment in these cells stain blue with Prussian blue stain (Perl's stain).

MORPHOLOGY

Gross

- Lung is **heavy**.
- Cut section (c/s) **rusty brown color** (due to hemosiderin pigment), **firm in consistency** (due to fibrosis) → known as **brown induration of lung**.

Microscopy (Fig. 5.1)

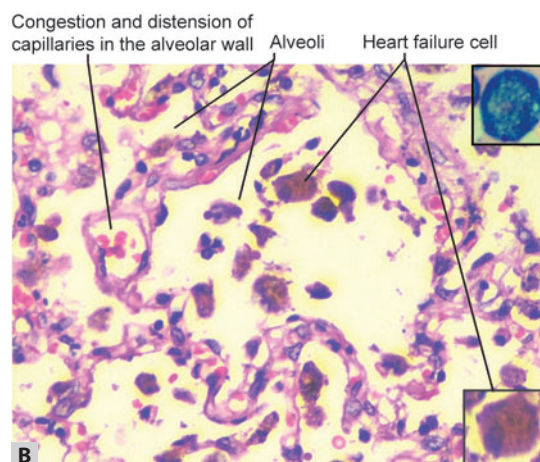
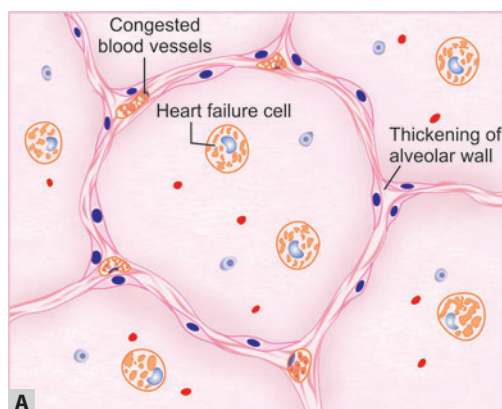
- **Distension and congestion of capillaries** in the alveolar septa of lung.
- **Thickened alveolar septa** due to increase in the fibrous connective tissue → responsible for the firm consistency of the lung.
- **Heart failure cells** are seen in the alveoli.

Hemosiderin-laden macrophages are known as heart failure cells. The term heart failure does not mean that these are seen in heart failure.

Chronic Venous Congestion of Liver

Q. Write short note on causes, gross and microscopic features of chronic venous congestion of liver/ CVC liver/nutmeg liver.

CVC liver: Nutmeg liver.



Figs 5.1A and B: Chronic venous congestion lung (A. diagrammatic and B. Hematoxylin and eosin) with thickened alveolar walls and hemosiderin laden macrophages (heart failure cells) in the alveolar lumen. Inset of B, lower right-hemosiderin laden macrophage and upper left-Perl's stain imparting blue-black color to hemosiderin in the cytoplasm

Causes

- **Right-sided heart failure** is the most common cause.
- **Rare:** Constrictive pericarditis, tricuspid stenosis and obstruction of inferior vena cava and hepatic vein.
- **Mechanism:** Dilatation of central veins → transmission of increased venous pressure to the sinusoids → dilatation of sinusoids → ischemic necrosis of hepatocytes in the centrilobular region.

MORPHOLOGY

Gross

- Liver increases in size and weight and the capsule appears tense.
- Cut section shows **alternate** (combination of) **dark and light areas** (Fig. 5.2) and resembles cross-section of a nutmeg (nutmeg liver).



Fig. 5.2: Gross appearance of chronic venous congestion of liver, which shows alternate dark and light area and resembles the cut surface of a nutmeg (inset)

- **Congested centrilobular regions** (with hemorrhage and necrosis) appear **dark red-brown**. Congestion is most prominent around terminal hepatic venule (central veins) within hepatic lobules.
- **Periportal** (better oxygenated) **region** of the lobules appear **pale** and may show fatty change.

Microscopy (Fig. 5.3)

- **Centrilobular region:**
 - **Congestion and hemorrhage** in the central veins (terminal hepatic venule) and adjacent sinusoids.
 - The **severe central hypoxia** may produce **centrilobular hepatocyte necrosis**.
 - **Thickening of central veins and fibrosis** in prolonged venous congestion.
 - **Cardiac sclerosis/cardiac cirrhosis** may occur with sustained chronic venous congestion (e.g. due to constrictive pericarditis or tricuspid stenosis).
- **Periportal region:** It shows **fatty change** in hepatocytes.

CVC liver if sustained for long time: Cardiac sclerosis/cardiac cirrhosis develops.

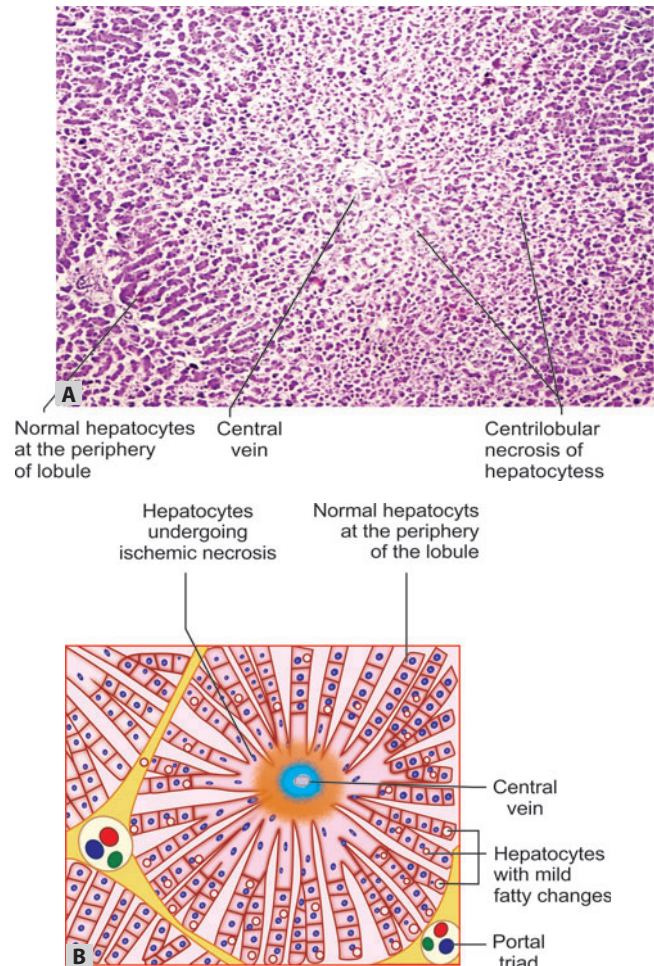
Congestive Splenomegaly (CVC Spleen)

Q. Write short note on CVC spleen/congestive splenomegaly.

Congestion and enlargement of spleen is called as congestive splenomegaly.

Causes

- Chronic obstruction to the outflow of venous blood from spleen leads to higher pressure in the splenic vein.



Figs 5.3A and B: Chronic venous congestion liver shows centrilobular necrosis with degenerating hepatocytes surrounded by apparently normal hepatic parenchyma in the periportal region. (A) Photomicrograph; (B) Diagrammatic

- **Intrahepatic obstruction to blood flow: Cirrhosis of the liver** is the main cause (e.g. alcoholic cirrhosis, pigment cirrhosis).
- **Extrahepatic disorders:**
 - ♦ **Systemic or central venous congestion:** For example tricuspid or pulmonic valvular disease, chronic cor pulmonale, right heart failure or following left-sided heart failure. Splenomegaly is only moderate and rarely exceeds 500 g in weight.
 - ♦ **Obstruction of the extrahepatic portal vein or splenic vein:** Due to spontaneous portal vein thrombosis, which is usually caused by intrahepatic obstructive disease, or inflammation of the portal vein (pylphlebitis). Thrombosis of the splenic vein can also develop by infiltrating tumors arising in neighboring viscera, such as carcinomas of the stomach or pancreas.

MORPHOLOGY**Gross****Q. Write short note on Gamna-Gandy bodies.**

- Spleen is **enlarged, firm and tense**. In long-standing chronic splenic congestion, spleen is markedly enlargement (1000–5000 g). **Capsule is thickened**.
- Cut section oozes dark blood.
- May show **Gamna-Gandy bodies**, which consist of **iron-containing, fibrotic, and calcified foci of old hemorrhage**.
- Enlarged spleen **may show excessive functional activity** termed as **hypersplenism** → leads to hematologic abnormalities (e.g. thrombocytopenia pancytopenia).
CVC spleen: Hypersplenism.

Microscopy

- **Red pulp**
 - **Dilatation and congestion** in the early stages.
 - Hemorrhage and fibrosis in later stages.
 - **Capillarization of sinusoids** may occur, in which sinusoids get converted into capillaries.
- **Thickened fibrous capsule and trabeculae**.
- **Slowing of blood flow** from the cords to the sinusoids → prolongs the exposure of the blood cells to macrophages in the spleen → leads to **excessive destruction of blood cells (hypersplenism)**.

Gamna-Gandy bodies: Iron-containing, fibrotic, and calcified foci of old hemorrhage.

Gamna-Gandy bodies contains:

- Hemosiderin (Perl's stain positive)
- Calcium (Von Kossa stain positive).

EDEMA**Q. Define edema.**

Definition: An abnormal accumulation of fluid in the interstitial space within tissues is called edema.

Edema: Excess fluid in the interstitial spaces within tissues.

Special forms of edema are listed in Table 5.1.

TABLE 5.1: Special forms of edema

Terminology	Body cavity involved
Hydrothorax	Pleural cavity
Hydropericardium	Pericardial cavity
Hydroperitoneum (ascites)	Peritoneal cavity

Types of Edema Fluid**Q. Tabulate the differences between transudate and exudate.**

The edema fluid may be either transudate or exudate. The differences between transudate and exudate are presented in Table 2.3.

1. **Transudate:** It is protein-poor fluid caused by increased hydrostatic pressure or reduced plasma protein.
 - **Causes:** Transudate is observed in heart failure, renal failure, hepatic failure, and certain forms of malnutrition.
2. **Exudate:** It is **protein-rich fluid** produced due to increased vascular permeability and is seen in **inflammation**.

Transudate is a protein-poor and cell-poor fluid. Exudate is protein-rich and cell-rich fluid.

Pathophysiologic Categories of Edema (Table 5.2)**Q. Define different pathophysiological categories of edema.**

Edema may be localized or generalized in distribution.

TABLE 5.2: Pathophysiologic categories of edema

Mechanism	Causes
Increased hydrostatic pressure	Impaired venous return <ul style="list-style-type: none"> • Generalized <ul style="list-style-type: none"> – Congestive heart failure – Constrictive pericarditis • Regional <ul style="list-style-type: none"> – Ascites in cirrhosis – Obstruction (e.g. thrombosis) or compression of veins (e.g. external mass) – Arteriolar dilation: Heat
Decreased plasma osmotic pressure (hypoproteinemia)	Nephrotic syndrome Ascites in cirrhosis of liver Malnutrition Protein-losing gastroenteropathy
Lymphatic obstruction	Inflammatory Neoplastic Postirradiation Postsurgical
Inflammation	Acute and chronic inflammation, angiogenesis
Sodium retention	Excessive salt intake with renal insufficiency Increased tubular reabsorption of sodium: e.g. increased renin-angiotensin-aldosterone secretion

Local/Localized Edema

- It is **limited to an organ or part** (e.g. arm, leg, epiglottis, larynx).
 - Obstruction of vein or lymphatic:** For example edema of limb (usually the leg) develops due to venous or lymphatic obstruction caused by **thrombophlebitis, chronic lymphangitis, resection of regional lymph nodes, filariasis**, etc.
 - Inflammation:** It is the **most common cause** of local edema.
 - Immune reaction:** For example urticaria (hives), or edema of the epiglottis or larynx (angioneurotic edema).

Generalized Edema

- It is **systemic** in distribution and affects visceral organs and the skin of the trunk and lower extremities.
 - Causes:** Disorder of fluid and electrolyte metabolism.
 - Heart failure
 - Nephrotic syndrome (renal diseases with massive loss of serum proteins into the urine)
 - Cirrhosis of the liver.

Anasarca: Severe form of generalized edema.

Inflammatory carcinoma of breast: Local lymphedema due to invasion and obstruction of subcutaneous lymphatics by tumor cells.

- The movement of water and salts between the intravascular and interstitial spaces is controlled mainly by two opposite effect of **Starling forces**.
- The force that drives the fluid out of circulation is **vascular hydrostatic pressure** and the force which drives the fluid into circulation is **plasma colloid osmotic pressure**.

Normal Fluid Balance

- Normally, the fluid flows out from the arteriolar end of the microcirculation into the interstitium.
- This is balanced by flowing in of the fluid at the venular end.
- A small amount of fluid, which may be left in the interstitium, is drained by the lymphatic vessels, and it reaches the bloodstream via the thoracic duct.

Mechanism of Edema

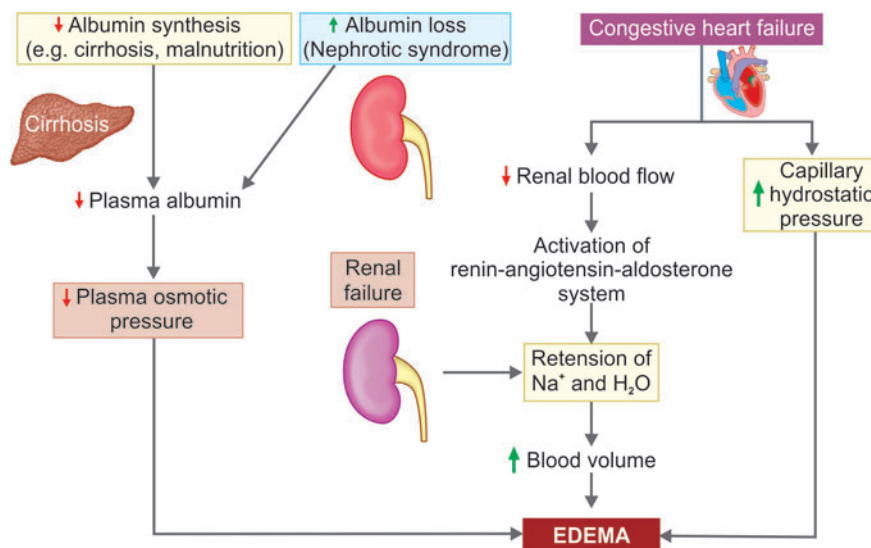
- Any mechanism, which interferes with the normal fluid balance, may produce edema.
- Increased capillary hydrostatic pressure or decreased colloid osmotic pressure** produces increased interstitial fluid.
- If the movement of fluid into tissues (or body cavities) exceeds lymphatic drainage, the fluid accumulates in the interstitial tissue.
- These mechanisms may operate singly or in combinations.

Mechanism/Pathogenesis of Edema (Fig. 5.4)

Q. Discuss the pathogenesis of edema in cirrhosis.

Increased Hydrostatic Pressure

Hydrostatic pressure at the capillary end of microcirculation drives the fluid out of the capillary into the interstitial tissue space. Any conditions, which increase the hydrostatic



Mechanism of edema

1. Increased hydrostatic pressure
2. Decreased colloid osmotic pressure
3. Sodium retention
4. Lymphatic obstruction.

Generalized edema: Most common cause is congestive heart failure.

Fig. 5.4: Pathogenesis of systemic edema from congestive heart failure, renal failure, or reduced plasma osmotic pressure

pressure, can produce edema. The increased hydrostatic pressure **may be regional or generalized**.

- **Local increase in hydrostatic pressure:** It can be due to **local impairment in venous return**. Examples,
 - **Deep venous thrombosis** in a lower extremity may produce localized edema in the affected leg.
 - **Postural edema** may be seen in the feet and ankle of individuals who stand in erect position for long duration.
- **Generalized increase in hydrostatic pressure:** It produces generalized edema. Most common cause is **congestive heart failure (CHF)**.
 - Congestive heart failure may be failure of the left ventricle, right ventricle or both.
 - **Right-sided heart failure** results in pooling of blood on the venous side of the circulation → increases the hydrostatic pressure in the venous circulation → increases movement of fluid into the interstitial tissue spaces → shows characteristic **peripheral pitting edema**.
 - **Left-sided heart failure** results in increased hydrostatic pressure in the pulmonary circulation → produces **pulmonary edema**.

Decreased Plasma Osmotic Pressure

Q. Discuss the pathogenesis of renal edema.

Plasma osmotic pressure normally tends to draw the fluid into the vessels. The **plasma osmotic pressure is dependent on plasma proteins, mainly on albumin** (major plasma protein). Decreased plasma osmotic pressure may be due to:

- **Reduced albumin synthesis:** Occurs in **severe liver diseases** (e.g. cirrhosis) or **protein malnutrition** (due to decreased intake of proteins).
- **Loss of albumin:** May occur in the **urine or stool**. **Nephrotic syndrome** is an important cause of loss of albumin in urine. **Malabsorption and protein losing enteropathy** are characterized by loss of protein in the stool.

Consequences of decreased plasma osmotic pressure:

- **Decreased plasma osmotic pressure** → **increased movement of fluid from circulation into the interstitial tissue spaces** → **reduced intravascular volume** → **decreased renal perfusion** → activates **increased production of renin, angiotensin, and aldosterone** → results in **salt and water retention**.
- These mechanisms cannot correct the reduced plasma volume because the persistence of primary defect of decreased serum protein.

Nephrotic syndrome: Massive loss of albumin urine → decreased serum albumin → decreased plasma osmotic pressure → generalized edema.

Sodium and Water Retention

Increased **retention of sodium salt is invariably associated with retention of water**. Sodium and water retention may be a primary cause of edema.

- **Mechanism**
 - **Increased hydrostatic pressure** due to increased plasma volume
 - **Decreased plasma colloid osmotic pressure** due to dilution effect on albumin.
- **Causes:** May be primary or secondary
 - **Primary:** It is associated with disorders of kidney such as renal failure, glomerulonephritis.
 - **Secondary:** It develops in disorders that decrease renal perfusion, most important cause being congestive heart failure.

Q. Mention the mechanism of cardiac edema.

- **Mechanism of edema in congestive heart failure**
 - **Decreased cardiac output** → causes **decreased flow of blood to the kidney** → **activates the renin-angiotensin system** → **retention of sodium and water**.
 - Other adaptations also occur, which includes increased vascular tone and elevated levels of antidiuretic hormone (ADH).
- **Water retention by ADH mechanism**
 - ADH is released from the posterior pituitary, when there is reduced plasma volume or increased plasma osmolarity.
 - Primary retention of water can occur due to the increased release of ADH.
 - **Increased secretion of ADH** is seen in association with lung cancer and **pituitary disorders**.
 - This can lead to hyponatremia and cerebral edema.

Lymphatic Obstruction

Q. Write short note on localized edema.

Lymphatic obstruction causes impaired drainage of lymph and produces localized edema, called as **lymphedema**.

Causes of Lymphatic Obstruction

- **Chronic inflammation of lymphatics** associated with fibrosis: For example, lymphedema occurring at scrotal and vulvar region due to **lymphogranuloma venereum**.

- **Invasive malignant tumors:** For example, **lymphedema of breast** due to blockage of subcutaneous lymphatics by **malignant cells** gives rise to **orange skin (peau d'orange)** appearance to the involved region of skin in the breast.
- **Pressure over lymphatic drainage** from outside: For example, tumors obstructing thoracic ducts.
- **Damage by surgery/radiation:** Patients with breast cancer may develop severe edema of the upper arm as a complication of **surgical removal and/or irradiation of the breast** and associated axillary lymph nodes.
- **Parasitic infestations:** In **filariasis** (caused by *Wuchereria bancrofti*), the parasite may cause extensive obstruction of lymphatics and lymph node fibrosis. If the block is in the inguinal region, it can produce edema of the external genitalia and lower limbs (upper arm if axillary region is involved) which may be massive and resemble the leg of an elephant and is known as **elephantiasis**.
- **Hereditary disorder: Milroy's disease** is a hereditary disorder characterized by abnormal development of lymphatics. The edema may be seen in one or both lower limbs.

Through lymph, proteins in the interstitial space are returned to the circulation. So, **edema fluid** produced due to lymphatic obstruction has a **high protein** concentration. The increased protein content may **stimulate fibrosis** in the dermis of the skin and is responsible for the induration found in lymphedema.

Angioneurotic edema:

- Autosomal dominant
- Mediated by vasoactive peptides such as bradykinin
- Low levels or abnormal function of a regulatory complement protein in the plasma, C1 inhibitor (C1 INH deficiency).

Lymphatic edema: Fluid in edema has high protein content.

Lymphedema: Lymphatic obstruction after modified radical mastectomy, radiation and filariasis.

Peau d'orange appearance of skin in the carcinoma breast: Lymphedema of breast due to blockage of subcutaneous lymphatics by malignant cells.

MORPHOLOGY

Edema can be **easily detected on gross examination**. It may involve any organ or tissue, but is **most common in subcutaneous tissues, the lungs, and the brain**. Microscopically, edema appears as a clear space, which separates the extracellular matrix.

Generalized Edema

It is seen mainly in the subcutaneous tissues.

- **Subcutaneous edema:** It may be diffuse or more easily noticed in regions with high hydrostatic pressures. In most cases, the distribution of **edema is dependent on gravity** and is termed **dependent edema**. Thus, it is prominent in the legs when standing, and in the sacrum when recumbent. **If pressure is applied** by a finger over substantially edematous subcutaneous tissue, it **displaces the interstitial fluid and leaves a depression**. This sign is called as **pitting edema**.
- **Edema of renal origin:** It can **affect all parts of the body**. Initially, it is observed in **tissues with loose connective tissue matrix**, such as the **eyelids and scrotal region**. Edema in the eyelids is called **periorbital edema** and is a characteristic of severe renal disease.

Pitting edema in right heart failure is due to increased hydrostatic pressure.

Pitting edema in cirrhosis is due to reduced osmotic pressure.

Q. Write briefly on pulmonary edema.

Pulmonary Edema

- **Gross:** The **weight of lungs is increased** 2 to 3 times of normal weight. **Cut section** shows frothy, **blood-tinged fluid** (due to mixture of air, edema, and extravasated red cells) **oozing from the lung**.
- **Microscopy:** The edema fluid is seen in the alveolar septa around capillaries and reduces the diffusion of oxygen. Edema fluid present in the alveolar spaces favors bacterial infection.

Q. Write briefly on brain edema.

Cerebral Edema:

- It may be **localized or generalized**. In generalized edema, the brain is grossly swollen with **distended gyri and narrowed sulci**. The ventricular cavities are compressed and as the brain expands, it may herniate.

Clinical Consequences

They range from minimal effects to rapidly fatal effect.

- **Generalized subcutaneous tissue edema:** It indicates the presence of an underlying **cardiac or renal disease**. Severe subcutaneous edema may delay wound healing or the clearance of infection.
- **Pulmonary edema:** It is common and most commonly **caused by left ventricular failure**. Other causes include renal failure, acute respiratory distress syndrome, and pulmonary inflammation or infection.

- **Cerebral/brain parenchymal edema:** It is **life-threatening**. In severe brain edema, the brain substance may herniate (extrude) through the foramen magnum, or occlude the blood supply to the brainstem. Both conditions may damage the medullary centers and lead to death.
- **Myxedema:** It is a form of **non-pitting** edema involving skin of face and visceral organs **observed in hypothyroidism**. The edema is due to excessive deposition of glycosaminoglycans and hyaluronic acid, in skin, subcutaneous tissue, and visceral organs.
- **Papilledema:** **Swelling of the optic nerve head** is called as papilledema. The concentric increase in pressure encircling the optic nerve produces stasis of venous outflow which leads to swelling of the optic nerve head. The causes are:
 - Compression of the nerve (e.g. primary neoplasm of the optic nerve)
 - Raised cerebrospinal fluid pressure surrounding the nerve.

FUNCTIONS OF NORMAL ENDOTHELIUM

Endothelial cells play an important role in both homeostasis and thrombus formation. They have both anti-thrombotic and prothrombotic (procoagulant) properties. The balance between these two opposing endothelial properties determines the thrombus formation.

Ultrastructurally, endothelial cells contain Weibel Palade bodies.

Antithrombotic Properties

Normally, the endothelial cells have antiplatelet, anticoagulant and fibrinolytic properties which prevent thrombosis (and also coagulation) (Fig. 5.5).

Antiplatelet Effects

They prevent platelet adhesion and aggregation following mechanism:

- **Intact endothelium prevents adhesion of platelets** (and plasma coagulation factors) to the highly thrombogenic subendothelial ECM.
- **Production of inhibitors of platelet aggregation** by endothelial cells: These include: **prostacyclin** (PGI_2), **nitric oxide** (NO) and **adenosine diphosphatase** (which degrades adenosine diphosphate-ADP).

Anticoagulant Effects

The endothelium inhibits coagulation by following molecules:

- **Heparin-like molecules:** Found in the endothelium and exert their anticoagulant effect indirectly through **antithrombin III**. They inactivate thrombin and coagulation factors (Xa and IXa).
- **Thrombomodulin:** Present on the endothelial cells and binds to thrombin and activates protein C, which **inhibits clotting** by proteolysis of factor Va and VIIIa.
- **Tissue factor pathway inhibitor** (TFPI): Inhibits tissue factor/factor VIIa complexes.

Fibrinolytic Effects

Endothelial cells synthesize tissue-type plasminogen activator (*t-PA*) which degrades whenever a thrombi is formed.

Prothrombotic Properties

Endothelial cells may be damaged or activated by several ways. These include trauma, inflammation, infectious agents, hemodynamic forces, plasma mediators, and cytokines. The damaged or activated endothelial cells promote prothrombotic state by its platelet, procoagulant and antifibrinolytic effects.

Platelet Effects

- Endothelial damage **exposes the subendothelial thrombogenic extracellular matrix (ECM)** and allows adhesion of platelets from circulation to ECM.
- *von Willebrand factor* (*vWF*) is produced by normal endothelial cells is essential cofactor that helps platelet binding to matrix elements.

Procoagulant Effects

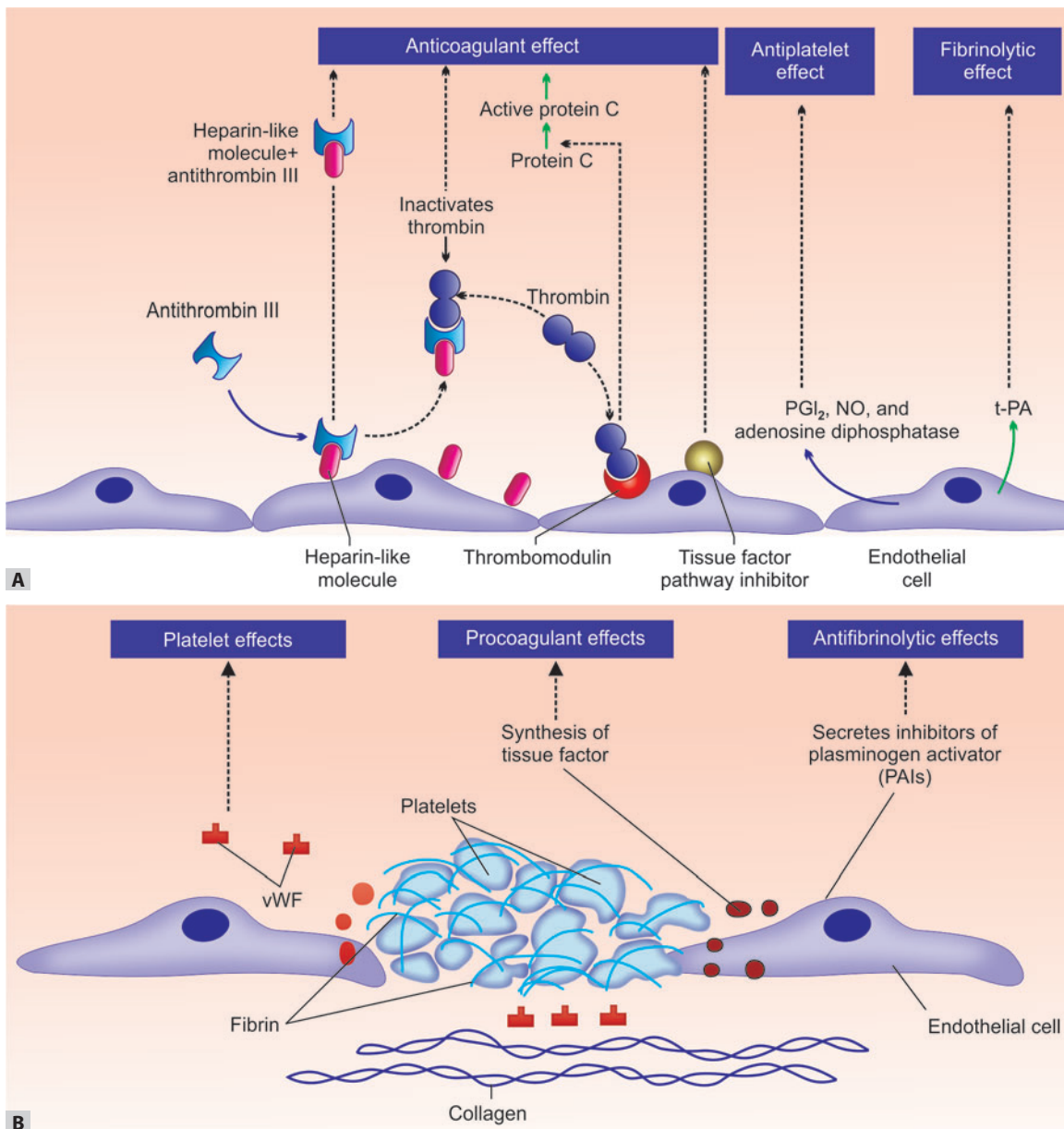
- Endothelial cells **synthesize tissue factor** in response to cytokines [e.g. tumor necrosis factor (TNF) or interleukin-1 (IL-1)] or bacterial endotoxin. Tissue factor activates the extrinsic coagulation cascade.
- Activated endothelial cells increases the catalytic function of activated coagulation factors IXa and Xa.

Antifibrinolytic Effects

Endothelial cells secrete inhibitors of plasminogen activator (PAIs). They reduce fibrinolysis and tend to favor thrombosis.

Intact, nonactivated endothelial cells inhibit thrombus whereas endothelial injury or activation promotes thrombus formation.

Antithrombotic and prothrombotic properties of endothelium are listed in Table 5.3.



Figs 5.5A and B: Endothelial factors that (A) inhibit and (B) favor thrombosis.

Abbreviations: PGI₂, prostacyclin; NO, nitric oxide; t-PA, tissue plasminogen activator; vWF, von Willebrand factor

THROMBOSIS

Q. Define thrombus.

Definition: Thrombosis is defined as the **process of formation of a solid mass in the circulating blood from the constituents of flowing blood.**

The **solid mass formed** is called as **thrombus** and it consists of an aggregate of coagulated blood containing platelets, fibrin, and entrapped cellular elements of blood.

Thrombosis: Formation of a solid mass from the constituents of flowing blood.

Etiology

Q. What is Virchow's triad?

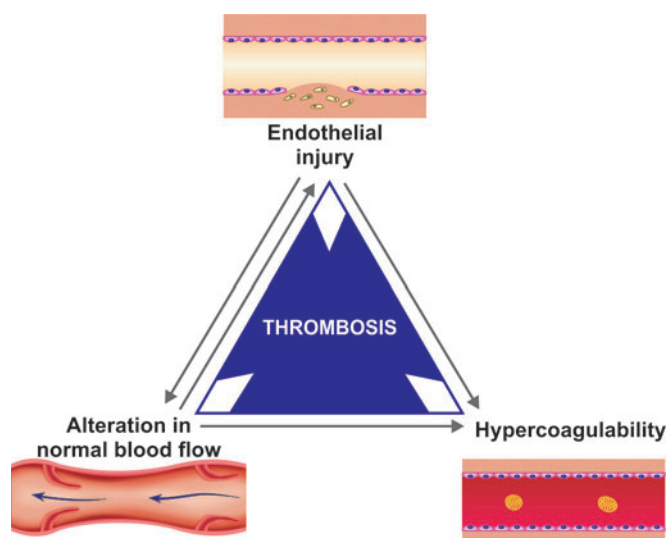
Q. Describe the etiopathogenesis of thrombus.

Three primary abnormalities can lead to formation of a thrombus and constitute Virchow's triad (Fig. 5.6). These include:

1. **Injury to endothelium** (changes in the vessel wall).
2. **Stasis or turbulent blood flow** (changes in the blood flow).

TABLE 5.3: Antithrombotic and prothrombotic properties of endothelium

Antithrombotic properties	Prothrombotic properties
Antiplatelet effects <ul style="list-style-type: none"> Acts as a barrier between platelets and subendothelial thrombogenic ECM. Produce inhibitors of platelet aggregation (e.g. PGI₂, NO and adenosine diphosphatase) 	Platelet effects <ul style="list-style-type: none"> Endothelial damage exposes the subendothelial thrombogenic ECM <i>von Willebrand factor</i> (vWF) produced by normal endothelial cells helps platelet binding to ECM
Anticoagulant effects <ul style="list-style-type: none"> Heparin-like molecules Thrombomodulin Tissue factor pathway inhibitor (TFPI) 	Procoagulant effects: <ul style="list-style-type: none"> Synthesis of tissue factor → activates the extrinsic coagulation cascade Activated endothelial cells increase the catalytic function of factors IXa and Xa
Fibrinolytic effect through tissue-type plasminogen activator (t-PA) → conversion of plasminogen to plasmin → cleaves fibrin.	Antifibrinolytic effects through secretion of inhibitors of plasminogen activator (PAIs) → reduce fibrinolysis.

**Fig. 5.6:** Virchow's triad in thrombosis. (1) Endothelial injury is the most important factor, (2) Alteration in blood flow (stasis or turbulence) and (3) Hypercoagulability

3. Hypercoagulability of the blood (changes in the blood itself).

Virchow's triad:

1. Endothelial injury
2. Abnormal blood flow
3. Hypercoagulability.

Injury to Endothelium (Changes in the Vessel Wall)

Endothelial injury may be either physical damage or endothelial dysfunction (or activation).

Physical Endothelial Injury

It is important for formation of thrombus in the **heart or the arterial circulation**. Normally, high flow rates in the heart and arterial circulation prevent adhesion of platelet to endocardium/endothelium and wash out any activated coagulation factors. The endothelial cell injury **promotes adhesion of platelets at the site of injury**.

Causes:

- **Heart:**
 - Chambers of heart: For example, endocardial injury due to myocardial infarction with damage to the adjacent endocardium, catheter trauma.
 - Valves: Small thrombi on the valves are called as vegetations.
 - ♦ Infective endocarditis: Thrombi on valves (e.g. mitral, aortic valve) damaged by a blood-borne bacteria or fungi
 - ♦ Damaged valves: For examples due to rheumatic heart disease, congenital heart disease
 - ♦ Libman-Sacks endocarditis in systemic lupus erythematosus
 - ♦ Nonbacterial thrombotic endocarditis: They are sterile vegetations on noninfected valves with hypercoagulable states.
- **Arteries:** For examples, ulcerated atherosclerotic plaques, traumatic or inflammatory vascular injury (**vasculitis**).
- **Capillaries:** Causes include **acute inflammatory lesions**, vasculitis and disseminated intravascular coagulation (DIC).

Mechanism:

- Physical loss of endothelium **exposes thrombogenic subendothelial ECM**.
- **Platelets** adhere to the site of endothelial injury and **release prothrombotic tissue factor**. There is local depletion of **antithrombotic factors** like PGI₂.

Endothelial Dysfunction

Definition: Endothelial dysfunction is defined as an **altered state, which induces an endothelial surface that is thrombogenic or abnormally adhesive to inflammatory cells**. Thus, thrombus can develop without any denudation or physical disruption of endothelium.

Causes: Hypertension, turbulent blood flow, toxins (e.g. bacterial endotoxins, toxins from cigarette smoke), radiation

injury, metabolic abnormalities (e.g. homocystinemia or hypercholesterolemia).

Mechanism: Endothelial dysfunction can **disturb the balance between prothrombotic and antithrombotic activities of endothelium by:**

- **Producing more procoagulant factors**, e.g. platelet adhesion molecules, tissue factor, PAIs or
- **Synthesizing less anticoagulant effectors**, e.g. thrombomodulin, PGI₂, t-PA.

Thrombosis: Can develop with physical injury to endothelium or endothelial dysfunction without physical injury.

Alterations in Normal Blood Flow

Normal blood flow is **laminar**, in which **platelets** (and other blood cellular elements) **flow centrally**, separated from endothelium by a slower moving layer of plasma.

Causes

- **Turbulence** (disturbed movement of blood): It can produce thrombus in the arteries and heart.
- **Stasis:** It is a **major cause for venous thrombosis**.

Venous thrombosis: Stasis is the major cause.

Mechanism

- Stasis and turbulence produce thrombus by the following mechanism:
 - **Promote endothelial injury/activation** and increases the procoagulant activity.
 - **Brings platelets into contact with the endothelium.**
 - **Prevent cleansing and dilution of activated clotting factors** by fresh flowing blood.
 - **Prevents flowing in of clotting factor inhibitors.**
- **Clinical disorder associated with turbulence and stasis:**
 - **Heart**
 - ◆ Acute myocardial infarction
 - ◆ Arrhythmias/atrial fibrillation: For example, rheumatic mitral stenosis in conjunction with disordered atrial rhythm (atrial fibrillation), it predisposes to mural thrombi in atria.
 - ◆ Dilated cardiomyopathy
 - **Arteries**
 - ◆ Ulceration of atherosclerotic plaques
 - ◆ Aneurysms: They cause local stasis.
 - **Veins:** Thrombi develop in the saphenous veins with varicosities or in deep veins.
- **Other causes**
 - **Hyperviscosity**, e.g. with polycythemia vera

- **RBC disorders**, e.g. **sickle cell anemia** can cause vascular occlusions and stasis.

Hypercoagulability

Definition: Hypercoagulability state (also known as thrombophilia) is defined as a **systemic disorder associated with increased tendency to develop thromboembolism**.

Causes: It is a less frequent cause of thrombosis. Causes can be divided into primary (genetic) and secondary (acquired) disorders (Box 5.1).

Secondary/acquired disorders (Table 5.5): The pathogenesis of acquired thrombophilia is usually multifactorial.

BOX 5.1: Major causes of hypercoagulable state

A. Primary (genetic)

Deficiency of antithrombotic (anticoagulant) factors

- Antithrombin III deficiency
- Protein C deficiency
- Protein S deficiency
- MTHFR gene mutation

Increased prothrombotic factors

- Activated protein C (APC) resistance (factor V mutation/ factor Va/ factor V Leiden)
- Excessive levels of prothrombin (prothrombin G20210A mutation)
- High levels of factors VII, XI, IX, VIII; von Willebrand factor; fibrinogen
- Homocystinuria

B. Secondary (acquired)

High-risk for thrombosis

- Prolonged bed rest or immobilization
- Myocardial infarction, atrial fibrillation
- Tissue injury (e.g. surgery, fracture, burn)
- Disseminated intravascular coagulation
- Cancer, prosthetic cardiac valves, heparin-induced thrombocytopenia
- Antiphospholipid antibody syndrome

Lower risk for thrombosis

- Nephrotic syndrome
- Hyperestrogenic states (pregnancy and postpartum), oral contraceptive use
- Cardiomyopathy, smoking, sickle cell anemia

Arterial thrombi: Seen in

Homocystinemia: Inherited or acquire disorder associated with both arterial and venous thrombosis.

When thrombosis develops in patient below the age of 50 years, genetic causes of hypercoagulability must be considered, even if there are acquired risk factors.

Hypercoagulability due to defective factor V gene is called Leiden mutation. It is the common inherited cause of hypercoagulability.

TABLE 5.4: Differences between arterial and venous thrombus

Characteristics	Arterial thrombus	Venous thrombus
Main cause	Injury to endothelium	Stasis
Rate of blood flow	Rapid	Slow
Usual type of thrombus	Mural	Occlusive
Common sites	Aorta, coronary, cerebral and femoral arteries	Superficial varicose veins and deep veins of leg
Gross		
Color	Gray-white	Red-blue
Lines of Zahn	More prominent	Less prominent
Composition	Friable meshwork of platelets, fibrin, RBCs and degenerating leukocytes	More trapped RBCs and relatively few platelets
Propagation	Retrograde manner from point of attachment of thrombus (i.e. towards heart)	In antegrade manner from point of attachment towards the direction of blood flow (i.e. towards the heart)
Effects	Ischemia causing infarction of area supplied by the artery containing thrombus	Thromboembolism, edema and ulceration
Aspirin: Prevents arterial thrombosis.		

Heparin and Warfarin: Prevents venous thrombosis.

Q. Antiphospholipid antibody syndrome.

Antiphospholipid syndrome: Associated with

- Venous thrombosis
- Recurrent abortion
- Antibody to lupus.

Terminology

Q. Write short note on mural thrombi.

- **Mural thrombus:** It is **attached to the wall** and projects into the lumen, **without complete occlusion of the lumen** (refer Figs 5.7B and 5.8). It occurs in heart chambers or in the aortic lumen.
- **Occlusive thrombus:** It **occludes the lumen of the blood vessel** (refer Fig. 5.8) and prevents the flow of blood. It usually occurs in veins or smaller or medium sized arteries.

Q. Write short note on vegetations.

- **Vegetation:** It is a **thrombus on heart valve** (refer Fig. 15.10) and appears as polypoid mass projecting into the lumen (e.g. infective endocarditis).

Mural thrombus: Occurs in heart chambers or in the aortic lumen.

Types of Thrombi

Thrombi may be **arterial or venous type**. Differences between arterial and venous thrombus are shown in Table 5.4.

MORPHOLOGY OF THROMBI

- **Layers in thrombus:**
 - **First layer** of the thrombus on the endothelium/endocardium is a **platelet layer**.
 - **On top of the platelet layer, fibrin** is precipitated to **form upstanding laminae** which anastomose to form an intricate structure which **resembles coral** (coralline thrombus). In **between the upstanding laminae** and anastomosing fibrin meshwork, the **red blood cells get trapped**. **Retraction of fibrin produces a ribbed appearance** on the surface of thrombus.

Q. Write briefly on lines of Zahn.

- **Lines of Zahn:** Both gross and microscopy of thrombus show **alternating light** (pale or white) area of platelets held together by fibrin, **and dark retracted area** of fibrin meshwork with trapped RBCs. These alternating laminations of light and dark are known as **lines of Zahn** (Fig. 5.7).

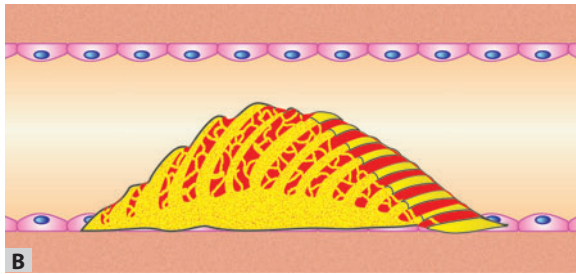
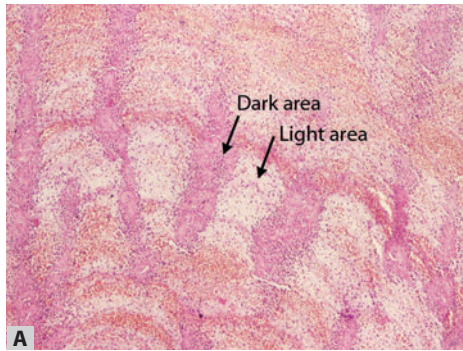
Lines of Zahn: They help to distinguish antemortem thrombus from postmortem clot.

Site and Types

Thrombi: Its size and shape depends on the site of origin and its cause. Thrombi can develop anywhere in the cardiovascular system.

- **Heart:**
 - **Cardiac thrombi:** Usually develops at sites of turbulence or endocardial injury.
 - ♦ More common in the atrial appendages.
 - ♦ Can also develop on the endocardial surface over the site of acute myocardial infarction (refer Fig. 15.8).
 - **Valves:** Thrombi on heart valves are called **vegetations** (refer Fig. 15.10). They are more common on **mitral or aortic valves**.

Rarely, a large round thrombus may form on the mitral valve and obstruct the lumen of the valve.



Figs 5.7A and B: Appearance of thrombus (A, microscopic and B, diagrammatic) showing alternating dark and light areas (lines of Zahn)

Thrombus: Lines of Zahn.

Agonal thrombi: Thrombi developing one or both ventricles shortly before death.

• Blood vessels:

- **Arteries:** Arterial thrombi tend to be **white**.
 - ◆ Aorta or larger arteries usually develop mural thrombi.
 - ◆ Thrombi developing in the medium or smaller arteries are frequently occlusive. They develop (in decreasing order of frequency) in the coronary, cerebral and femoral arteries.
- **Veins:**
 - ◆ Venous thrombosis (phlebothrombosis) are usually occlusive, and form a long cast of the lumen. They occur usually at sites of stasis, and contain more trapped RBCs (and relatively few platelets). They are therefore known as red, or stasis thrombi.

Venous thrombus: Deep vein of the lower extremity (90% of cases) is the commonest site.

Attachment: Thrombi are focally attached to the underlying surface.

Postmortem Clots

Q. Describe the appearance of postmortem clot.

Determination of whether a clot (antemortem thrombi) is formed during life or after death (postmortem clot) is

important in a medical autopsy and in forensic pathology. Differences between antemortem venous thrombi and postmortem clots are listed in Table 5.6. After death, the red blood cells settle and produce two layers.

- **Lower layer:** It contains **many RBCs**, which have settled by gravity → forms a dark red lower portion. This has a reddish and gelatinous appearance which resembles **currant jelly**.
- **Upper layer:** It is **poor in cells and is yellow-white**. It is firm representing coagulated plasma without red blood cells. It is called **chicken fat** because of its color and consistency.

Postmortem clot: Currant jelly and chicken fat appearance.

Q. Differences between postmortem clot and thrombi.

TABLE 5.5: Differences between antemortem venous thrombi and postmortem clots

Characteristics	Antemortem venous thrombi	Postmortem clots
Attachment to vessel wall	Focally and firmly attached	Not attached
Consistency	Dry, granular, firm and friable	Gelatinous, soft and rubbery
Shape	May or may not fit the vascular contours	Have the shape of the vessel in which it is found
Appearance	Alternate dark and white areas	Currant jelly or chicken fat appearance
Lines of Zahn	Present	Absent
Mechanism	Changes in blood flow (stasis) and hypercoagulability	Occurs in stagnant blood in which gravity fractionates the blood

Fate of the Thrombus (Fig. 5.8)

Q. Describe fate of a thrombus.

- **Dissolution/lysis** of thrombi without any consequences.
 - **Recent thrombi may totally disappear** due to activation of fibrinolysis.
 - **Old thrombi are more resistant to lysis.**
- **Propagation of thrombi:** It is the **process in which thrombi grow and increase in size**. The thrombus which was initially **mural**, may become **occlusive** thrombus. The propagating portion of a thrombus is poorly attached to the wall and therefore, **prone to fragmentation and embolization**.
 - **Arterial thrombi grow retrograde** from the point of attachment

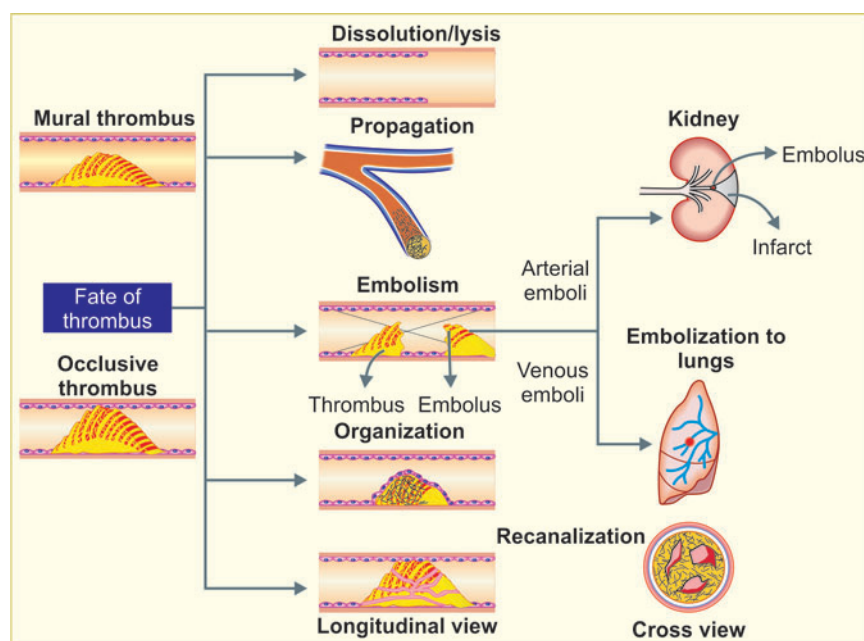


Fig. 5.8: Fate of thrombus

Thrombi: Treatment with fibrinolytic agents is effective only when it is administered in the first few hours of a thrombotic episode.

Fate of thrombus:

- Dissolution
- Propagation
- Embolization
- Organization
- Recanalization
- Mycotic aneurysm.

– Venous thrombi extend in the direction of blood flow.

- **Embolization:** Thrombi may get detached from its site of origin and form emboli. These emboli can travel to other sites through the circulation and lodge in a blood vessel away from the site of thrombus formation. The consequences depend on the site of lodgment. Large venous thrombi may get detached and travel to the pulmonary circulation to the lungs as pulmonary emboli.
- **Organization:** If thrombi are not dissolved (either spontaneously or by therapy), these older thrombi become organized by the ingrowth of endothelial cells, smooth muscle cells, and fibroblasts. Small, organized thrombi may be incorporated into the vessel wall.
- **Canalization/recanalization:** New lumen/channels lined by endothelial cells may form in an organized thrombus. These capillary channels may form thoroughfare channels and can re-establish the continuity of the original lumen.
- **Mycotic aneurysm:** Rarely, the central region of the thrombi may undergo enzymatic digestion due to lysosomal enzymes released from trapped leukocytes and platelets. If bacteremia develops, these thrombi may become infected and produce an inflammatory mass. This region of the vessel becomes weak and can produce mycotic aneurysm.

VENOUS THROMBOSIS (PHLEBOTHROMBOSIS)

Q. Write short note on phlebothrombosis and Discuss the causes and pathogenesis of venous thrombosis.

Veins Involved

Most commonly superficial or deep veins of the leg are involved.

- **Superficial venous thrombi**
- **Site:** They develop in the varicosities involving saphenous veins.
 - **Effects:** It can cause local congestion, swelling (edema), pain, and tenderness. The local edema and impaired venous drainage predispose the overlying skin to infections from slight trauma and to the development of varicose ulcers. Embolization is very rare.

Superficial venous thrombi:

- Varicose ulcers
- Predisposition to infection of the overlying skin
- Embolization very rare.
- **Deep venous thrombosis (DVT):** Lower extremity DVTs are found in association with venous stasis and hypercoagulable states.

- **Sites:** Larger veins in the leg at or above the knee (e.g. popliteal, femoral, and iliac veins).
- **Effects:**
 - ◆ Even though DVTs can cause local pain and edema, the venous block produced by them is usually rapidly balanced by the development of collateral channels.
 - ◆ More prone to embolization into the lungs and produce pulmonary infarction. About 50% of DVTs are asymptomatic and are detected after embolization.

Deep vein thrombosis: More prone to embolization to lungs.

Pathogenesis of DVT (Phlebothrombosis)

Q. Describe the causes and pathogenesis of venous thrombosis/phlebothrombosis.

Deep venous thrombosis is caused by the same etiological factors that favor arterial and cardiac thrombosis. These include endothelial injury, stasis, and a hypercoagulable state.

Different stages in the development of DVTs (Fig. 5.9) are:

- **Primary platelet thrombus**
 - Damage to the intima of the vein causes adhesion of platelets at damaged site → platelets aggregate to form **pale platelet thrombus**.
 - Venous stasis favors accumulation of coagulation factors, which is activated to form fibrin.

Primary platelet thrombus: Adhesion and aggregation of platelets at the site of intimal injury is similar to snowdrift during a snowstorm or flies sticking to an oily paper.

- **Coralline thrombus:** The fibrin and thrombin formed encourages further accumulation of platelets. The **platelets along with fibrin form upright laminae** growing across the stream. Between the laminae, stasis promotes further deposition of fibrin with trapped RBC and WBCs. This **produces alternate layers of fused platelets and fibrin with trapped blood cells**. The contraction of fibrin produces a **characteristic ribbed (ripple) appearance** on the surface of thrombus. These **raised platelet ridges are known as lines of Zahn**.

Coralline thrombus: Laminae anastomose to form a structure which resembles coral (sea weed).

- **Occluding thrombus:** Further growth of thrombus progressively **occludes the lumen** of the vein and forms occluding thrombus.

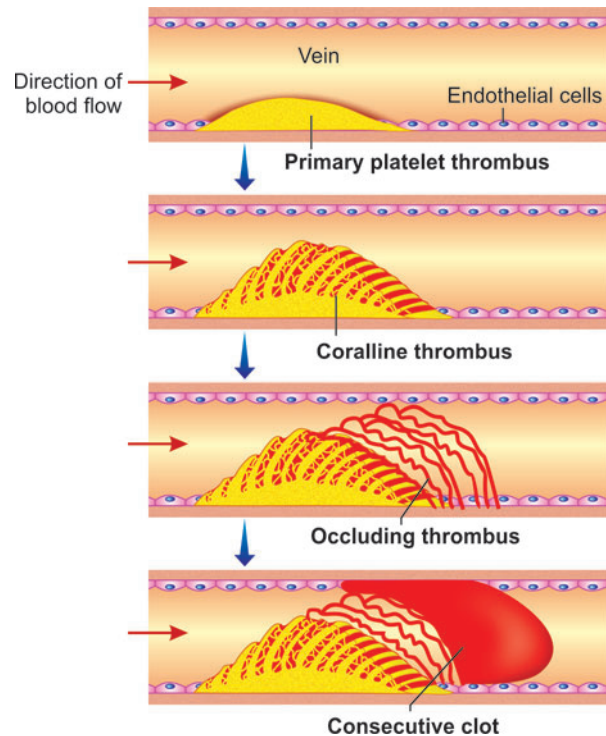
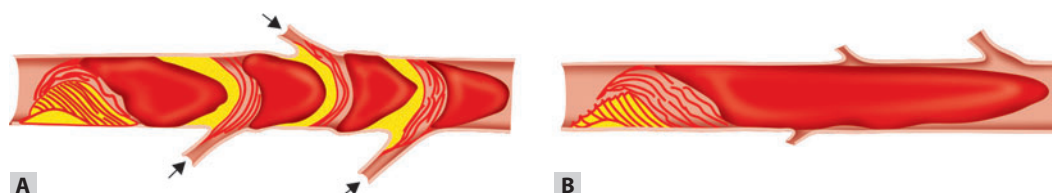


Fig. 5.9: Various stages in the pathogenesis of phlebothrombosis

- **Consecutive clot:** Occlusive thrombus **stops the blood flow**. Since, thrombi can develop only in the streaming blood, the **blood column beyond the occluding thrombus clots to form a consecutive clot**. Thereafter, the consecutive clot may be halted and endothelialized or it can spread (**propagate**).
- **Propagated clot:** There are **two methods** of propagation (Fig. 5.10):
 - **Thrombus formation in each tributary:** The consecutive clot when reaches the entrance of venous tributary may form another coralline thrombus over the clot. This causes occlusion of opening of tributary. A consecutive clot will again form up to the opening of next venous tributary. Thus, **several thrombi with associated consecutive clot** may be formed.
 - **Clotting en mass beyond the thrombus:** Another method of propagation is **formation of long column of consecutive clot** attached to only one thrombus. These consecutive clots may break and produce fatal massive pulmonary embolism.

Homan sign: Forced dorsiflexion of the foot produces tenderness in the calf when there is DVT.



Figs 5.10A and B: Propagation of venous thrombi.

(A) Thrombus formation in each tributary; (B) Clotting en mass beyond the thrombus

Thrombophlebitis

Inflammation of the wall of vein causes damage to the endothelium and **may lead to thrombus formation**. The thrombus formed is firmly attached to the wall of the vein and do not embolize. Sterile inflammation may be produced by direct trauma, chemicals or ionizing radiation. Bacterial inflammation of veins may be produced in the veins near the infected areas.

- **Thrombophlebitis migrans (migratory thrombophlebitis or Trousseau syndrome)**
 - **Characterized by recurrent thrombotic episodes** involving the superficial and deep veins, especially of the extremities.
 - May develop as a **complication of deep-seated cancers** such as cancer of pancreas (tail and body), lung, stomach, and female genital tract.
 - First described by Trousseau who had pancreatic cancer, when he noticed it on himself and suggested that it is a **sign of visceral cancer**. It is known as Trousseau's syndrome.

Migratory thrombophlebitis:

- Known as Trousseau's syndrome
- Recurrent venous thrombosis
- Complicates deep-seated cancers (e.g. pancreas, lung).

Consequences of Thrombi

It depends on the site of the thrombosis.

- **Obstruction of involved vessel:** Thrombi can cause obstruction of involved arteries and veins.

- **Arterial thrombi:** They may cause **infarctions** in the region supplied by the involved vessel. Occlusion at a certain locations (e.g. a coronary artery) can be life-threatening.
- **Venous thrombi:** Small venous thrombi may cause no symptoms. Larger thrombi can cause **congestion and edema in region distal to obstruction** by thrombus. Forced dorsiflexion of the foot produces tenderness in the calf associated with DVT and is known as **Homan sign**.
- **Embolization:** Arterial, cardiac and venous thrombi can undergo fragmentation and detach to form emboli. It is the major complication and these are thromboemboli. The consequences of embolism depends on: (1) site of lodgement of emboli, (2) tissue affected and (3) source of thromboemboli.
 - **Arterial and cardiac thromboemboli:** The commonest sites of lodgment of emboli are the brain, kidneys, and spleen because of their rich blood supply. The various effects are mentioned in pages 98-100.
 - **Venous emboli:** They may lodge in the lungs causing various consequences of **pulmonary embolism** (refer pages 98-99).

Conditions associated with both arterial and venous thrombi are listed in Table 5.6.

Complications of arterial and cardiac thrombi:

- Fragmentation and embolization
- Common sites of embolization: Organs with rich blood supply, i.e. brain, kidneys, and spleen.

TABLE 5.6: Conditions associated with both arterial and venous thrombi

• Homocysteinuria	• Antiphospholipid antibody	• Hyperhomocysteinemia
• Disseminated intravascular coagulation (DIC)	• Heparin-induced thrombocytopenia	• Essential thrombocythemia
• Cancer	• PNH	• Polycythemia vera
		• Dysfibrinogenemia

EMBOLISM

Q. Define embolism.

Definition: An embolus is a **detached intravascular solid, liquid, or gaseous mass** that is **transported in the blood to a site distant from its point of origin**.

Embolus: Detached intravascular mass transported to a site distant from its point of origin.

Example, **prostatic carcinoma metastasis to the spine**. It occurs through retrograde spread via intraspinal veins which carry the emboli from large thoracic ducts and abdominal veins due to increased pressure in the body cavities (e.g. during coughing or straining).

Unless otherwise specified, emboli should be considered thrombotic in origin and the process is known as thromboembolism.

Types of Emboli

Q. Mention different types of emboli.

Classification: Depending on:

1. **Physical nature of the emboli:**
 - **Solid: Thromboemboli**, atheromatous material, **tumor emboli**, tissue fragments, bacterial clumps or parasites, foreign bodies.
 - **Liquid: Fat**, bone marrow and amniotic fluid.
 - **Gaseous: Air** or other gases.
2. **Whether infected or not**
 - **Bland:** Sterile.
 - **Septic:** Infected.
3. **Source (Fig. 5.11):** The emboli may be **endogenous** (form within the body) or **exogenous** (introduced from outside).
 - **Cardiac emboli:** Usually they arise from left side of the heart. Example, emboli from: (1) atrial appendage, (2) left ventricle in **myocardial infarction**, (3) vegetations on the valves in **infective endocarditis**.
 - **Vascular emboli:**
 - **Arterial emboli:** For example, atheromatous plaque, aneurysms.
 - **Venous emboli:** For example, deep vein thrombus, tumor emboli.
 - **Lymphatic emboli:** For example, tumor emboli.

4. Flow of emboli.

Q. Write short note on paradoxical embolism.

- **Paradoxical emboli:** They are rare and the **emboli originate in the venous circulation and bypass the lungs** by traveling **through a right-to-left shunt** such as an atrial septal defect (incompletely closed/patent foramen ovale) or interventricular defect. Then, they **enter the left side of the heart and block the blood flow to the systemic arteries**.

Q. Write short note on retrograde embolism.

- **Retrograde emboli:** Emboli, which **travel against the flow of blood** are known as retrograde emboli.

PULMONARY EMBOLISM

Q. Write short note on pulmonary embolism.

Definition: Pulmonary embolism (PE) is defined as an **embolism in which emboli occlude pulmonary arterial tree**.

Site of Origin of Emboli (Fig. 5.11)

- **Deep leg veins:** DVTs are the source in more than 95% of cases of pulmonary emboli. Deep leg veins include **popliteal, femoral or iliac veins**.
- **Other sites:** Pelvic veins, vena cava.

Risk of pulmonary embolism: Major risk factor is after surgery. The risk increases with advancing age, obesity,

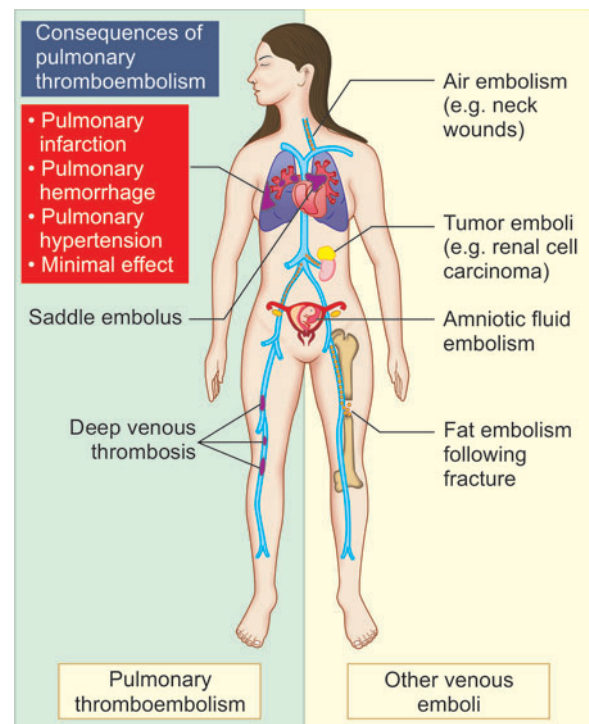


Fig. 5.11: Sources and effects of venous emboli

prolonged operative procedure, postoperative infection, cancer, and pre-existing venous disease.

Mechanism: DVTs undergo fragmentation → these thromboemboli are carried through progressively larger vascular channels → into the right side of the heart → right ventricle → they enter into the pulmonary arterial vasculature.

Pulmonary thromboembolism: Majority of the cases the source is femoral veins.

Fate of Pulmonary Embolism

Fate depends on the size of the embolus.

1. **Resolution or organization:** Small pulmonary emboli may travel into the smaller, branches of pulmonary arteries and may **resolve completely**. Most (60–80%) of them are clinically **silent**. With passage of time they become **organized** and are incorporated into the wall of pulmonary vessel.
2. **Massive pulmonary embolism:** When emboli **obstruct 60% or more of the pulmonary circulation**, it is known as massive pulmonary embolism.

Q. Write short note on saddle embolism.

- **Saddle embolus:** It is a **large pulmonary embolus** which **lodges at the bifurcation of the main pulmonary artery**. It produces acute massive obstruction of the blood flow to both lungs.
- **Effects:**
 - **Acute right ventricular failure.**
 - **Shock:** Right ventricular failure → reduction in left ventricular cardiac output → sudden severe hypotension (or shock) → may result in sudden death.
- 3. **Multiple recurrent pulmonary emboli:** These may **fuse to form a single large mass**. Usually, the patient who has had one PE is likely to have recurrent emboli.
- 4. **Paradoxical embolism:** (refer page 98).

Paradoxical embolism: Embolus passes through an interatrial/interventricular defect and gains access to the systemic circulation.

Consequences (Fig. 5.11)

1. **Pulmonary infarction:**
 - Most (about 75%) small pulmonary emboli do not produce infarcts. However, an embolus can **produce infarction in the patients with congestive heart failure or chronic lung disease**.

- **Gross:**
 - **Type:** Usually **hemorrhagic type, because of blood supply to the infarcted (necrotic) area by the bronchial artery**.
 - **Shape:** **Pyramidal** in shape with the base of the pyramid on the pleural surface. When the blood in the infarcted area is resorbed, the center of the infarct becomes pale.
 - **Fate:** Granulation tissue grows from the edges of the infarct results in organization of infarct and forms a **fibrous scar**.
- **Clinical features:** Cough, stabbing pleuritic pain, shortness of breath, and occasional hemoptysis. Pleural effusion is a common complication and pleural fluid is often blood stained.

Pulmonary infarction: It is rare, because lung has a dual blood supply by the bronchial arteries and the pulmonary artery.

Pulmonary embolism: Only 10% of emboli cause pulmonary infarction.

Pulmonary infarct:

- Uncommon in the young
 - About 3/4 affect lower lobes
 - Pyramidal in shape with apex pointing toward the hilus of the lung.
2. **Pulmonary hemorrhage:** Obstruction of medium-sized pulmonary arteries by emboli and subsequent rupture of these vessels can **result in pulmonary hemorrhage**.
 3. **Pulmonary hypertension:** Multiple recurrent pulmonary emboli → may cause **mechanical blockage of the arterial bed** → **result in pulmonary hypertension** → **right ventricular failure**.
 4. **Minimal effect:** Obstruction of **small end-arteriolar branches** of pulmonary artery by emboli usually **neither produces hemorrhage nor infarction**.

Pulmonary embolism: Patient who has had one PE is at a high-risk of developing another one.

SYSTEMIC THROMBOEMBOLISM

Definition: It is defined as an embolism in which **emboli occlude systemic arterial circulation**.

Systemic arterial embolism **usually produces infarcts** in the region supplied by the involved vessel.

Sources of Systemic Emboli (Fig. 5.12)

- **Heart:** Most common source of thromboemboli.
 - **Intracardiac mural thrombi:** Most common source. Examples:

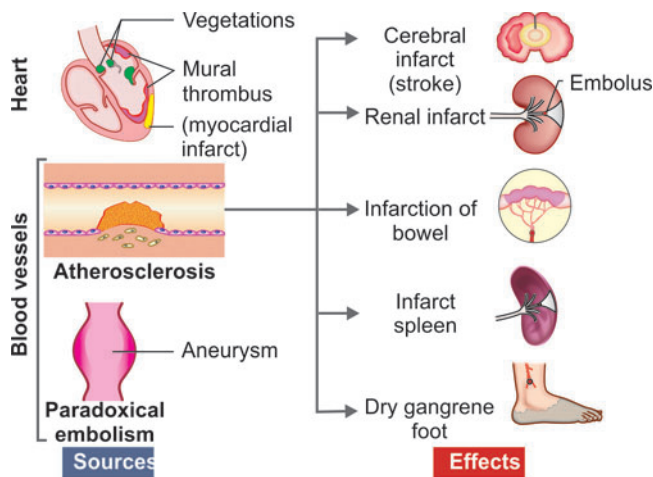


Fig. 5.12: Common sources and effects of systemic arterial emboli. It usually arises from the left side of the heart or from major arteries. Usual consequence is either infarction or gangrene at the site of lodgment

- ◆ Myocardial infarct of left ventricular wall
- ◆ In mitral stenosis, dilatation of left atrium and atrial fibrillation predisposes to thrombus and embolization.
- **Paradoxical emboli:** Rare source
- **Valvular source:** Examples, **bacterial endocarditis** (valvular vegetation from aortic or mitral valves) or prosthetic valves
- **Blood vessels:** Thrombi on **ulcerated atherosclerotic plaques** or from **aortic aneurysms**
- **Unknown origin.**

Systemic thromboembolism: Majority of the cases the source is left side of the heart.

Source of cardiac mural thrombi:

1. Myocardial infarction of left ventricle (2/3)
2. Left atrial dilation and fibrillation (1/4).

Consequences

- The arterial emboli can travel to a wide variety of sites. This is in contrast to venous emboli, which lodge mainly in one vascular bed namely the lung.
- The arterial emboli tend to pass through the progressively narrow arterial lumen and lodge at points where the vessel lumen narrows abruptly (e.g. at bifurcations or in the area of an atherosclerotic plaque).
- **Fate of thromboembolus at the site of arrest:**
 - **Propagation and obstruction:** Thromboemboli may grow (propagate) locally at the site of arrest and

produce severe obstruction leading to **infarction of the affected tissues** (Fig. 5.12).

- **Fragmentation and lysis.**

Major Sites Affected by Arterial Thromboemboli (Fig. 5.12)

1. **Lower extremity (75%):** Embolism to an artery of the leg may produce **gangrene**.
2. **Brain:** Arterial emboli to the brain may produce **ischemic necrosis** in the brain (strokes).
3. **Intestine:** Emboli in the mesenteric vessels may produce **infarction of the bowel**.
4. **Kidney:** Renal artery embolism may cause small peripheral **infarcts** in the kidney.
5. **Blood vessels:** Emboli originating from bacterial vegetation may cause inflammation of arteries and produce **mycotic aneurysm**.
6. **Other sites:** Spleen and upper extremities are less commonly affected.

FAT AND MARROW EMBOLISM

Q. Describe fat embolism.

Fat and marrow embolus consists of **microscopic globules of fat with or without bone marrow** elements. Release of these elements into the circulation produces fat embolism.

Causes

- **Trauma to adipose tissue with fracture:** Severe trauma to adipose tissue, particularly accompanied by **fractures of bone** release fat globules or fatty marrow (with or without associated hematopoietic marrow cells) into ruptured blood vessels. Fat embolism occurs in about 90% of individuals with **severe skeletal injuries**, but less than 10% of them have clinical findings.
- **Soft tissue trauma and burns.**
- **During vigorous cardiopulmonary resuscitation.**

Fat embolism: Commonly develop following fracture of long bones.

Manifestation

In **most of the cases** it is **asymptomatic**. Sometimes, it may manifest as potentially **fatal fat embolism syndrome**.

Fat embolism syndrome: It is the term applied when the patients develops **symptoms due to severe fat embolism**. It develops in only minority of patients.

Pathogenesis

Fat embolism syndrome involves both mechanical obstruction and biochemical injury.

- **Mechanical obstruction:**

- Trauma to adipose tissue associated with **fracture releases** emboli consisting of **fat globules and/fatty marrow**. These fat microemboli along with red cell and platelet aggregates may **enter the capillaries** which are ruptured at the site of the fracture.
- The trauma may also cause hemorrhage into the marrow and into the subcutaneous fat. This increases interstitial pressure above capillary pressure, and fat globules are forced into the circulation.
- The emboli travel through the circulation and can **occlude the pulmonary and cerebral microvasculature**.

- **Biochemical injury:**

- The chemical composition of the fat present in the lung in fat embolism is different from that in adipose tissue. The mechanical obstruction alone cannot explain this difference. So, pathogenesis probably involves mechanical obstruction associated with biochemical injury.
- **Biochemical injury is produced by free fatty acids** that are released from the fat globules. Free fatty acids **produce local toxic injury to endothelium**. They cause platelet activation and granulocyte recruitment along with release of injurious free radical, protease, and eicosanoid. These biochemical injury increases the severity of the vascular damage produced by mechanical obstruction.

Fat embolism: Fatty acids from fat globules produce local toxic injury to endothelium.

Consequences of Fat Embolism

It depends on the size and quantity of fat globules and whether the emboli are arrested in the pulmonary or systemic circulation. The paradoxical fat emboli may reach systemic circulation (e.g. through patent foramen ovale) and gets deposited in brain, kidney, etc.

- **Sites of arrest of fat emboli:**

- Emboli in the venous side lodge **in the lungs**.
- **If emboli pass into systemic circulation**, they may be arrested in **brain, kidneys** and other organs.

- **Autopsy findings:** Numerous **fat globules** can be found impacted **in the microvasculature** of the lungs (in pulmonary emboli) and brain and sometimes other organs (in systemic emboli).

- **Lung:** The lungs typically show the changes of **acute respiratory distress syndrome**.

- **Brain:** The lesions include **cerebral edema, small hemorrhages**, and occasionally microinfarcts.

- **Demonstration of fat embolism:** Fat is dissolved during routine tissue preparations by the solvents (xylene/xylol) used in paraffin embedding. The microscopic demonstration of fat microglobules requires frozen sections and special stains for fat (e.g. Sudan III and IV, Oil Red O, and osmic acid).

Special stains for fat: Sudan III, Sudan IV, Oil Red O, and osmic acid.

Clinical Presentation

The most severe form of fat embolism syndrome may be fatal.

- **Time of development:** It develops **1 to 3 days after the traumatic injury**.
- **Respiratory symptoms:** These include sudden onset of **tachypnea, dyspnea and tachycardia** which may lead to **respiratory failure**.
- **Neurologic symptoms:** These include **irritability, restlessness, delirium and coma**.
- **Hematological findings:**
 - **Thrombocytopenia:** Rapid onset of thrombocytopenia produces **diffuse petechial rash** (found in 20%–50% of cases) and may be a useful **diagnostic** feature.
 - **Anemia:** It is due to aggregation of red cells and/or due to hemolysis.
- **Chest radiography:** It shows diffuse opacity of the lungs → may progress to an **opacification of lungs** (whiteout)-characteristic of **acute respiratory distress syndrome**.

Anemia in fat embolism: Due to aggregation of RBCs and hemolysis.

Fat embolism syndrome-clinical features: Dyspnea, petechial rash, irritability and restlessness.

Fat embolism: Fatal only in 10% of cases.

Thrombocytopenia in fat embolism: Due to platelet adhesion by fat globules.

AIR EMBOLISM

Q. Write short note on air/gas embolism/Caissons disease/decompression sickness.

Air embolism occurs when **air is introduced into venous or arterial circulation**.

Causes

- **Trauma/injury:** Air may enter the venous circulation through **neck wounds and chest wall injury**.
- **Surgery/invasive procedures:** These include invasive surgical procedures such as **thoracocentesis**, punctures of the great veins during **obstetric or laparoscopic procedures**, into the **coronary artery during bypass surgery**, cerebral circulation by neurosurgery in the “sitting position”, or hemodialysis.
- **Criminal abortion.**

Amount of air required: It is usually **more than 100 cc** to have a clinical effect of air embolism.

Mechanism: In the circulation, air/gas bubbles tend to coalesce to form frothy masses → which physically obstruct vascular blood flow in the right side of the heart.

Microscopy: Air bubbles are seen as empty spaces in capillaries and small vessels of the lung/brain.

Air embolism: More than 100 cc of air is required to have clinical effect.

- **Musculoskeletal system:** Small vessel obstruction → reduced blood supply to skeletal muscles and supporting tissues in and about joints → produces muscular and joint pain → patient doubles up in pain. This painful condition is called the **bends**.
- **Respiratory system:** Obstruction of blood vessels of the lungs causes edema, hemorrhage, and focal atelectasis or emphysema. This may lead to a form of respiratory distress called the **chokes**.
- **Nervous system:** It may cause coma or even death.

Nitrogen has an affinity for adipose tissue. Hence, obese individuals are at increased risk of developing decompression sickness.

Treatment of acute decompression sickness is by placing the individual in a high pressure chamber. This will force the gas bubbles back into solution.

Decompression sickness: Bends and chokes-nitrogen gas bubbles occlude lumen of blood vessels.

Decompression Sickness

It is a form of gas embolism and may be acute or chronic.

Acute Decompression Sickness

Cause: It develops when individuals **exposed to sudden decrease in atmospheric pressure**. Risk factors include:

- Individuals when exposed to high atmospheric pressure, such as **scuba and deep-sea divers and underwater construction workers** (e.g. tunnels, drilling platform construction), during rapid ascent to low pressure.
- Individuals in **unpressurized aircraft** during rapid ascent.
- **Sport diving.**

Mechanism

- When air is breathed at **high atmospheric pressure** (e.g. during a deep-sea dive), **large amounts of inert gas** such as nitrogen or helium are **dissolved in the blood, body fluids and tissues**.
- When the individual **ascends gradually**, the dissolved gas (particularly nitrogen) comes out from solution in the blood and tissues and exhaled. **It does not produce any injury.**
- However, **if ascent is too rapid, gas bubbles form in the blood circulation and within tissues** → obstruct the flow of blood → injure the cells.

Effects

The gas bubbles within small vessel obstruct the blood supply bends and chokes.

Chronic Decompression Sickness

Caisson Disease

- A chronic form of decompression sickness is known as **Caisson disease** (named for the pressurized vessels/diving bells used in the bridge construction).
- Workers in these pressurized vessels may develop both acute and chronic forms of decompression sickness.
- Characteristic features: **Avascular necrosis:** Gas embolus in vessel produces obstruction to blood flow → causes multiple foci of **ischemic (avascular) necrosis of bone**. The more commonly involved bone includes the head of the femur, tibia, and humerus.

AMNIOTIC FLUID EMBOLISM

Q. Write short note on amniotic fluid embolism.

Amniotic fluid embolism **develops when amniotic fluid along with fetal cells and debris enter the maternal circulation**. The entry occurs through open (ruptured) uterine and cervical veins or a tear in the placental membranes (Fig. 5.13).

Time of occurrence: It is a rare maternal threatening complication, which occurs **at the end of labor and the immediate postpartum period**.

Consequences: From the venous circulation, amniotic fluid emboli enter the right-side of the heart and finally rest in pulmonary circulation. Amniotic fluid has a high thromboplastin activity and initiates a potentially **fatal disseminated intravascular coagulation (DIC)**.

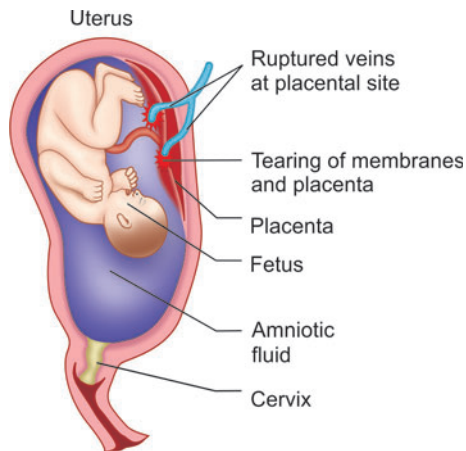


Fig. 5.13: Amniotic fluid embolism

MORPHOLOGY

- **Amniotic fluid contents within pulmonary vasculature:** Amniotic fluid emboli are composed of squamous cells shed from fetal skin, lanugo hair, fat from vernix caseosa, and mucin derived from the fetal respiratory or gastrointestinal tract.
- **Other findings:** These include marked pulmonary edema, diffuse alveolar damage, and features of DIC.

Clinical Features

- **Abrupt onset:** It develops during immediate postpartum period, and is characterized by sudden onset of severe dyspnea, cyanosis, and neurologic impairment ranging from headache to seizures. Patient develops shock, coma and death.
- **Bleeding:** If the patient survives the initial acute crisis, patient develops bleeding due to disseminated intravascular coagulation (DIC).
- **Acute respiratory distress syndrome.**

Amniotic fluid embolism: Abrupt onset of dyspnea, hypotension and bleeding due to DIC—at the end of labor or immediate postpartum period.

MISCELLANEOUS PULMONARY EMBOLI

- **Foreign bodies**
 - **Talc emboli:** It may occur in intravenous drug abusers who use talc as a carrier for illicit drugs.
 - **Cotton emboli:** It may occur due to cleansing of the skin by cotton prior to venipuncture.
- **Schistosomiasis:** The ova of schistosoma may gain entry into the circulation from bladder or gut and lodge in the lungs.

- **Tumor emboli:** It may occur during hematogenous dissemination of cancer.
- **Atheromatous emboli (cholesterol emboli):** Fragments of atheromatous plaque may embolize.
- **Platelet emboli:** During early stages of atherosclerosis, there is platelet deposition in the intimal surface of blood vessels. They may form platelet emboli.
- **Infective emboli:** In infective endocarditis, the vegetations seen on the diseased heart valves may become infected. These infected vegetations may break off and form infective emboli. Their effects are due to both emboli and infective agent that may weaken the wall of the vessel → may lead to the formation of a 'mycotic' aneurysm. Mycotic is a misnomer because the infective agent is usually bacterial, not fungal.

Mycotic aneurysm: Mycotic is a misnomer because the infective agent is usually bacterial, not fungal.

INFARCTION

Q. Define Infarct.

Definition: An infarct is a **localized area of ischemic necrosis** caused by occlusion of either the arterial blood supply or the venous drainage. The **process** of producing infarct is known as **infarction**.

Infarct: Localized area of ischemic necrosis caused by occlusion of either the arterial blood supply or the venous drainage.

Mostly infarct is coagulative type of necrosis due to sudden occlusion of arterial blood supply. If the patient survives, the infarct heals with a scar.

Common and important infarcts are shown in Table 5.7.

Causes of Infarction

Q. What are the causes of red and pale infarct?

- **Arterial causes:** Most important
 - **Occlusions of lumen:** It is the most common cause and may be due (1) **thrombus** or (2) **embolus** (Fig. 5.12).

TABLE 5.7: Common and important infarcts

Organ/tissue affected	Infarction
Heart	Myocardial infarction
Brain	Cerebral infarction
Lung	Pulmonary infarction
Bowel /intestine	Intestinal infarct
Extremities	Gangrene

- **Causes in the wall:** For example, local vasospasm, **hemorrhage into an atheromatous plaque** or thromboangiitis obliterans
- **External compression of vessel:** Tumor.
- **Venous causes**
 - **Occlusions of lumen** may be due (1) thrombus or (2) embolus
 - **Extrinsic vessel compression:** Tumor, torsion of a vessel (e.g. in testicular torsion or bowel volvulus), **strangulated hernia**.

Venous thrombosis: Infarcts caused by venous thrombosis usually occur in organs with a single efferent vein (e.g. testis and ovary).

Although venous thrombosis can cause infarction, mostly it produces congestion.

Factors that Determine the Outcome of an Infarct

Q. Mention the factors that influence the development of an infarct.

The outcome of vascular occlusion may range from no or minimal effect to the death of a tissue or individual. The **major factors** that determine the outcome of infarct are:

1. **Nature of the vascular supply:**
 - **Dual/parallel blood supply:** Organs or tissues with double or parallel blood supply are **less likely to develop infarction**, e.g. lung, liver, hand and forearm.
 - **End-arterial blood supply:** Kidney and spleen has blood supply, which are end-arteries with little or no collaterals. Obstruction of vessels in these organs usually causes tissue death and **infarction**.
2. **Rate of occlusion:** **Slow occlusion is less likely to produce infarction than rapid occlusion.** This is because it provides time to develop alternate perfusion pathways.
3. **Vulnerability of tissue to hypoxia:**
 - **Neurons are highly sensitive to hypoxia.** They undergo necrosis even if the blood supply is occluded for 3 to 4 minutes.
 - In heart, myocardial cells are also quite sensitive to hypoxia, but less sensitive than neurons. Myocardial cells die after only 20 to 30 minutes of ischemia.
4. **Oxygen content of blood:** In a normal individual, partial obstruction of a small vessel may not produce any effect, but in a patient with anemia or cyanosis same may produce infarction.

Classification (Table 5.8)

Q. Write short note on different types of infarcts, their causes and common sites of occurrence.

TABLE 5.8: Classification of infarct

According to color	Presence or absence of infection	According to the age of infarct
<ul style="list-style-type: none"> • White/pale (anemic) • Red (hemorrhagic) 	<ul style="list-style-type: none"> • Septic, when it is infected • Bland, when it is free of infection 	<ul style="list-style-type: none"> • Recent or fresh • Old or healed

Q. Mention the organs involved in red and pale infarct.

White/Pale Infarcts

They occur:

- With **arterial occlusions**
- In **solid organs**
- With **end-arterial circulation without a dual blood supply** (e.g. heart, spleen, and kidney)
- **Tissue with increased density** which prevents the diffusion of RBCs from adjoining capillary beds into the necrotic area.

Red/Hemorrhagic Infarcts

They occur:

- With **venous occlusions**, e.g. ovary.
- In **loose textured tissues**, e.g. lung: They allow red cells to diffuse through and collect in the necrotic zone.
- In **tissues with dual blood supply**, e.g. lung and small intestine: It allows blood flow from an unobstructed parallel blood supply into a necrotic zone.
- In **tissues previously congested** due to decreased venous drainage.
- When **blood flow is re-established to a site of previous arterial occlusion** and necrosis, e.g. following coronary angioplasty of an obstructed coronary artery.

White/pale infarct:

- Heart
- Kidney
- Spleen.

Hemorrhagic infarct: seen in

- Ovary
- Lung
- Small intestine.

In **red/hemorrhagic infarcts** there is bleeding into the necrotic area from adjacent arteries and veins which is not observed in pale infarct.

MORPHOLOGY

White/pale Infarcts

Q. Write short note on organs involved in pale and red infarcts.

Organs involved includes **heart, kidneys, spleen**, and dry gangrene of the extremities.

Gross:

- Usually **wedge-shaped** (Fig. 5.14).
- **Occluded blood vessel** is seen **at the apex** and the **periphery/surface** of the organ **forms the wide base**.
- **Acute infarcts** are **poorly defined** and **slightly hemorrhagic**.
- After 1 to 2 days, the infarct becomes soft, sharply demarcated, and light yellow in color.
- **Margins of infarct appear well-defined** because of narrow rim of congestion caused by inflammation.
- **As time passes**, infarcts progressively become **paler** and more **sharply defined**.

White infarct: Wedge-shaped with occluded vessel at the apex and periphery of the organ forms the base.

Red/Hemorrhagic Infarcts

- Organs with a double blood supply: e.g. **lung, liver**
- Organs with extensive collateral circulation: e.g. **small intestine and brain**
- Reperfusion of infarcted area: e.g. red infarct may occur in heart when the infarcted area is reperfused

Gross: Appear as **sharply circumscribed** area of necrosis, **firm** in consistency and **dark red to purple** in color.

Microscopy of Infarct

- **Both pale and red infarct** characteristically shows **ischemic coagulative necrosis**.
- Microscopic changes of **frank necrosis** appear after about **4 to 12 hours**.
- **Acute inflammation cells** infiltrate the necrotic area from the viable margins all-round the infarcts within a few hours. It becomes **prominent within 1 to 2 days**.
- Followed by a **reparative process**, which begins **at the preserved margins**. The **necrotic cells** in infarcts and extravasated red cells are **phagocytosed by macrophages**.
- In tissues composed of stable or labile cells, **parenchymal regeneration** can occur at the periphery where stromal architecture is preserved.
- **Granulation tissue** may **replace the infarcted area** which **matures to form scar tissue**.
- If the infarct is large (e.g. in heart or kidney), the necrotic center may persist for months.

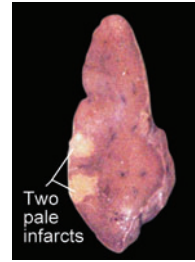


Fig. 5.14: Infarct spleen showing two wedge shaped pale/white infarct

White/pale infarct:

- Heart
- Kidney
- Spleen.

In contrast to other organs, the central nervous system infarction shows **liquefactive necrosis**. The necrotic focus may become cystic and filled with fluid and is referred to as a cystic infarct.

Septic infarctions: They may occur in two situations:

- **Infection:** Infarct may get infected when it is **seeded by pyogenic bacteria**, e.g. infection of pulmonary infarct.
- **Septic emboli:** They contain organisms and can produce septic infarct, e.g. vegetations of bacterial endocarditis may cause septic infarct of spleen.

The organisms present in a **septic infarct** convert infarct into a frank **abscess**.

SHOCK

Q. Define shock.

Introduction: Shock is the most common, important, and very serious medical condition. It is the final common pathway for several clinical events, which are capable of causing death. These events include severe hemorrhage, extensive trauma or burns, large myocardial infarction, massive pulmonary embolism, and severe microbial sepsis.

Definition: Shock is a **pathological process** that results from inadequate tissue perfusion, **leading to cellular dysfunction and organ failure**.

Characteristic features: **Extreme and widespread failure of the circulatory system** (either due to decreased cardiac output or reduced effective circulating blood volume) → **systemic hypotension** (either due to reduced cardiac output or to reduced effective circulating blood volume) → **life-threatening inadequate/impaired tissue perfusion (hypoperfusion)** → tissue hypoxia → **reversible cellular injury** → **irreversible tissue injury and organ failure** → **death**.

Q. List the main types of shock with suitable examples.

TABLE 5.9: Major types of shock

Types of shock	Principal mechanisms	Clinical example
Hypovolemic shock		
Loss of blood/plasma/ fluid → decreased circulating blood volume → low cardiac output → hypotension, and shock	Loss of blood volume	Massive hemorrhage, trauma
	Loss of plasma volume	Massive burns
	Loss of fluid	Vomiting, diarrhea, severe gastroenteritis
Cardiogenic shock		
Direct myocardial damage or a mechanical abnormality of the heart → low cardiac output → reduced cardiac output and blood pressure	Myocardial damage	Myocardial infarction Myocarditis Ventricular rupture
	Mechanical	Valvular failure (stenosis or incompetence) Hypertrophic cardiomyopathy Ventricular septal defect
	Arrhythmic	Ventricular arrhythmias
Septic shock	Endothelial activation/injury; leukocyte-induced damage, activation of cytokines, and disseminated intravascular coagulation	Overwhelming microbial infections (bacterial, fungal, viral, rickettsial)
Others		
Neurogenic shock	Result of loss of vascular tone and peripheral pooling of blood	Anesthetic accident or a spinal cord injury
Anaphylactic shock	Acute widespread systemic vasodilation and increased vascular permeability results in tissue hypoperfusion and hypoxia	IgE-mediated hypersensitivity reaction

Common sources of infection associated with septic shock: Pneumonia, peritonitis, pyelonephritis, abscess (especially intra-abdominal), primary bacteremia, etc.

Shock: Pathological process due to inadequate tissue perfusion.

Classification

Q. Classify shock.

According to etiology (cause) shock can be classified into three major general categories (Table 5.9).

Etiology and Pathogenesis

Q. Describe the etiology and pathogenesis of shock.

Q. Write short note on hypovolemic shock.

Hypovolemic Shock

Hypovolemic shock results from low cardiac output due to:

- **Loss of blood:** For example, massive hemorrhage.

- **Loss of plasma:** For example, severe burns.
- **Loss of fluid:** Vomiting, diarrhea, severe gastroenteritis, e.g. cholera.

Inadequate blood or plasma volume and fluid loss → hypovolemia → low cardiac output → hypotension → inadequate perfusion of tissue.

Hypovolemic shock: Most commonly due to blood loss.

Cardiogenic Shock

Q. Write short note on cardiogenic shock.

Cardiogenic shock results from low cardiac output due to:

- **Intrinsic myocardial damage:** For example, massive myocardial infarction, ventricular arrhythmias.
- **Extrinsic pressure or compression of heart:** For example, cardiac tamponade.
- **Obstruction to the outflow blood from ventricles:** For example, pulmonary embolism.

The various causes of cardiogenic shock produce → severe dysfunction of left ventricle → **decreases cardiac output** → decreased tissue perfusion of tissue. The left-sided heart failure also reduces the entry of blood from pulmonary vein into the left atrium. This leads to movement of fluid from pulmonary vasculature into the pulmonary interstitial space and into the alveoli resulting in **pulmonary edema**.

Cardiogenic shock: Most commonly due to acute **myocardial infarction**.

Septic Shock

Q. Describe the pathogenesis of septic shock.

Definition: Septic shock is defined as **shock due to severe sepsis with hypotension, which cannot be corrected by infusing fluids**.

Septic shock results from vasodilation and peripheral pooling of blood and is associated with dysfunction of multiple organs distant from the site of infection.

Septic shock: Due to severe sepsis with hypotension.

Causative organisms

- Septic shock may be caused by **Gram-positive** (most common) or **Gram-negative bacteria, fungi**, and, very rarely, protozoa or Rickettsiae. Hence, the older term “endotoxic shock”, is not appropriate.
- The common gram-positive bacteria include *Staphylococcus aureus*, *enterococci*, *Streptococcus pneumoniae*, and gram-negative bacilli which are resistant to usual antibiotics.

Organisms causing septic shock:

- Gram positive: *Staphylococcus aureus*, *enterococci*, *Streptococcus pneumoniae*
- Gram negative resistant to usual antibiotics.

Major Pathogenic Pathways in Septic Shock

- **Trigger:** Most of septic shocks are triggered by bacteria or fungi that normally do not produce systemic disease in immunocompetent hosts.
- **Hallmark of septic shock:** It is **tissue hypoperfusion** due to **decrease in peripheral vascular resistance** as a result of **systemic vasodilation** and **pooling of blood in the periphery**. Cardiac output may be normal or even increased in early stages.
- **Initiation of shock:** Several **microbial constituents** can initiate the pathogenesis of septic shock. These constituents **and mediators produced by host** act in an incompletely known way to produce septic shock.

Pathogenesis of Septic Shock

- **Major factors** contributing to the pathogenesis of septic shock (Fig. 5.15) are: (1) Inflammatory and counter-inflammatory responses, (2) endothelial cell activation and injury, (3) induction of a procoagulant state, (4) metabolic abnormalities, (5) organ dysfunction and (6) immune suppression.

Septic shock: Microbial components activate both innate and adaptive immunity. The activated inflammatory cells produce inflammatory mediators.

Septic shock: Microbial constituents or inflammatory mediators cause endothelial cell activation.

Septic/endotoxic shock: Initiating mechanism is endothelial injury/activation.

Septic shock consequences of endothelial activation:

- Activation of thrombosis
- Increased vascular permeability
- Vasodilation.

Septic shock: Multiorgan failure such as kidneys, liver, lungs and heart.

Toxic shock syndrome is similar to septic shock and is produced by a group of microbial exotoxins called superantigens.

Metabolic abnormalities in septic shock:

- Insulin resistance
- Hyperglycemia
- Decreased glucocorticoid production.

Inflammatory and Counter-inflammatory Responses

Triggering of proinflammatory response:

- Through activation of receptors on cells of the innate immune system
 - **Engagement of receptors on cells:** In sepsis, various microbial components of cell wall (e.g. bacterial peptides) engage receptors present on cells of the innate immune system (e.g. Toll-like receptors-TLRs).
 - **Release of proinflammatory mediators:** These receptors on activation trigger **production of pro-inflammatory mediators** such as TNF, IL-1, IFN- γ , IL-12, IL-18 and cytokine-like mediators such as high mobility group box 1 protein (HMGB1). They also produce reactive oxygen species and lipid mediators such as prostaglandins and platelet activating factor (PAF).

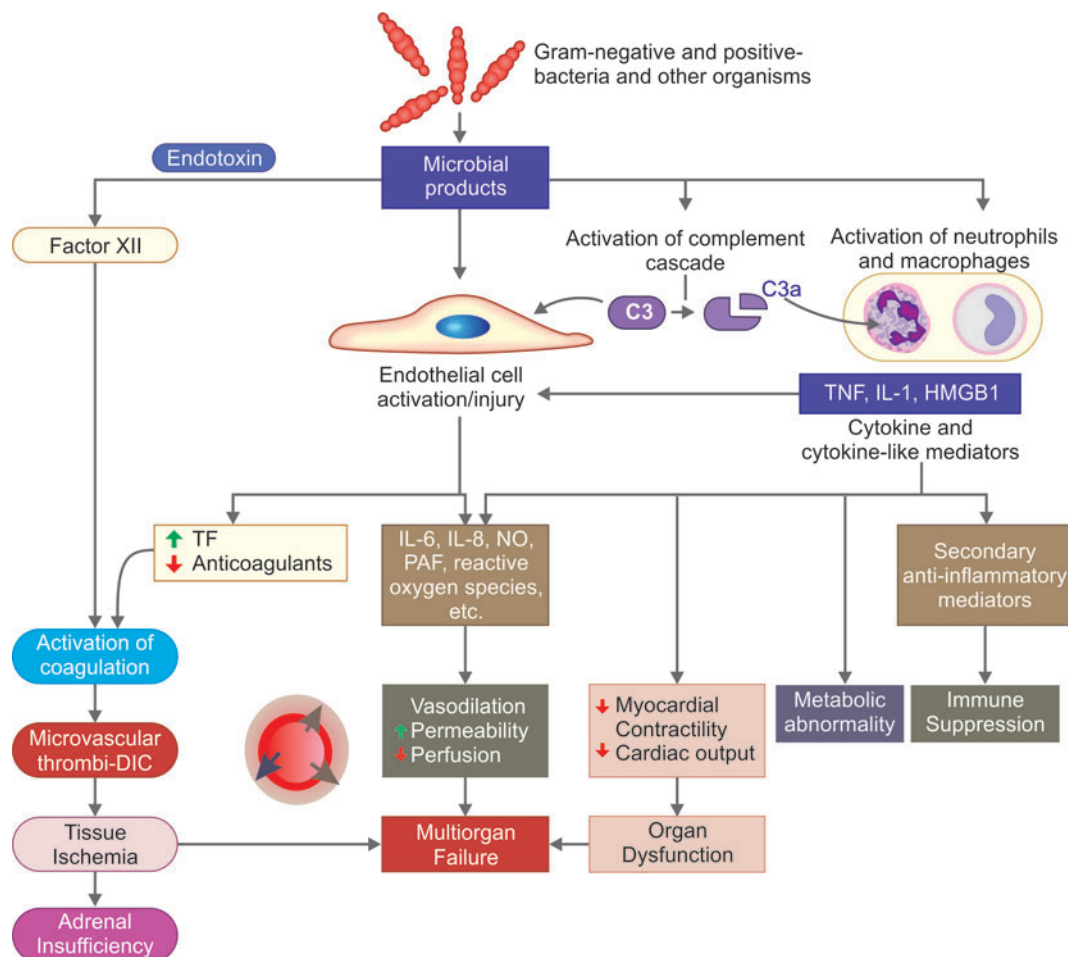


Fig. 5.15: Pathogenesis of septic shock. Microbial products initiate endothelial cell activation/injury activates endothelial cells, complement activation, activation of neutrophils and macrophages, factor XII. These initiating events lead to end-stage multiorgan failure.

Abbreviations: DIC, disseminated intravascular coagulation; HMGB1, high mobility group box 1 protein; NO, nitric oxide; PAF, platelet activating factor; TF, tissue factor; TF, tissue factor

- **Effect of inflammatory mediators:** These pro-inflammatory effector molecules activate endothelial cells (and other cell types) to upregulate expression of adhesion molecule. This in turn stimulates production of cytokine and chemokine.
- **Activation of complement cascade:** It also occurs due to microbial components, resulting in the production of anaphylotoxins (C3a, C5a), chemotactic fragments (C5a), and opsonins (C3b). All these complement products contribute to the proinflammatory state.
- **Activation of coagulation:** Microbial components can also activate coagulation directly through factor XII and indirectly through altered endothelial function.

Activation of counter-regulatory immunosuppressive mechanisms:

- The hyperinflammatory state produced by sepsis also activates counter-regulatory immunosuppressive

mechanisms. This involves both innate and adaptive immune cells. Thus, in a patient with sepsis, there may be oscillation between hyperinflammatory and immunosuppressed states.

- **Mechanisms for the immune suppression:** These include a shift from pro-inflammatory (TH1) to anti-inflammatory (TH2) cytokines, production of anti-inflammatory mediators (e.g. soluble TNF receptor, IL-1 receptor antagonist, and IL-10) and lymphocyte apoptosis.

Endothelial Activation and Injury

- Endothelial cell activation/injury is caused by either microbial constituents or proinflammatory state (leukocyte-derived inflammatory mediators).
- Inflammatory cytokines cause loosening of endothelial cell tight junctions. This causes widespread vascular

leakage of protein-rich fluid from vessels into the interstitial tissue resulting in the accumulation of edema fluid throughout the body.

- Edema has injurious effects on both supply of nutrient and removal of waste. This impairs tissue perfusion and may be exacerbated by attempts to support the patient with intravenous fluids.
- Endothelial activation also upregulates production of nitric oxide (NO) and other vasoactive inflammatory mediators (e.g. C3a, C5a, and PAF). These may cause relaxation of vascular smooth muscle and systemic hypotension.

Induction of a Procoagulant State

Factors activating coagulation system in sepsis:

- **Activation of factor XII** by microbial components such as **endotoxin**.
- **Pro-inflammatory cytokines (e.g. IL-6):**
 - They **increase the production of tissue factor** by monocytes and possibly endothelial cells.
 - Reduce the production of endothelial anticoagulant factors, such as tissue factor pathway inhibitor, thrombomodulin, and protein C.
 - Reduce fibrinolysis by increasing plasminogen activator inhibitor-1 expression.

Consequences of activation of coagulation system:

- This leads to systemic activation of thrombin and the deposition of fibrin-rich thrombi in small vessels, often throughout the body. This **produces dangerous complication DIC** in about 50% of septic patients. This compromises tissue perfusion formation. The consumption of coagulation factors and platelets leads to deficiencies of these factors and causes **bleeding and hemorrhage**.
- The vascular leak and tissue edema reduces the flow of blood flow in the small vessels, produces stasis and diminishes the clearing of activated coagulation factors.

Metabolic Abnormalities

- **Insulin resistance** and hyperglycemia: It is due to the action of pro-inflammatory cytokines such as TNF and IL-1, stress-induced hormones (e.g. glucagon, growth hormone, and glucocorticoids), and catecholamines. Hyperglycemia decreases neutrophil function, suppresses its bactericidal activity and causes increased expression of adhesion molecule on endothelial cells.
- **Decreased glucocorticoid production:** Initially, there is increased glucocorticoid production, and is later followed by decreased production due to adrenal insufficiency. **Adrenal necrosis may also develop due to DIC (Waterhouse-Friderichsen syndrome).**

- **Lactic acidosis:** Cellular hypoxia and diminished oxidative phosphorylation may produce increased lactate and lactic acidosis.

Organ Dysfunction

- **Decrease supply of oxygen and nutrients to the tissues:** Due to systemic hypotension, interstitial edema, and thrombi in the small vessels.
- **Decreased contractibility of myocardium and cardiac output:** It is due to increased levels of cytokines and secondary mediators. This along with increased vascular permeability and endothelial injury can lead to the **adult respiratory distress syndrome**.
- **Multiorgan failure:** Finally, above factors lead to failure of multiple organs, particularly the **kidneys, liver, lungs, and heart** resulting in death.

Immune Suppression

It occurs in patients with septic shock. It is probably **due to:**

- **Production of anti-inflammatory mediators** (e.g. soluble TNF receptor, IL-1 receptor antagonist, and IL-10).
- **Widespread apoptosis of lymphocytes.**

Toxic shock syndrome is similar to septic shock and is produced by a group of microbial exotoxins called superantigens.

Stages of Shock

Q. Describe 3 different /various stages of shock.

Shock is a progressive disorder, which if not treated, leads to death. It can be divided into three phases.

1. **Nonprogressive (compensated/reversible) phase:** During the initial phase, homeostatic compensatory mechanisms **redistribute the blood supply** in such a way that the effective **blood supply to the vital organs is maintained**. This is achieved by neurohumoral mechanisms, which try to maintain cardiac output and blood pressure.
 - Compensatory changes:** The neurohumoral mechanism produces the following compensatory changes:
 - **Widespread vasoconstriction** except vital organs. **Coronary and cerebral vessels usually maintain relatively normal blood flow, and oxygen delivery.** Cutaneous vasoconstriction → produces the coolness and pallor of the skin.
 - **Fluid conservation by kidney.**
 - **Tachycardia.**
2. **Progressive phase:**
 - **If the underlying causes are not corrected, shock passes to the progressive phase.**

- Characterized by **widespread tissue hypoperfusion** and **hypoxia** → intracellular aerobic respiration replaced by **anaerobic glycolysis** → **increased** production of **lactic acid** → metabolic **lactic acidosis** → **decreases** the tissue **pH** → dilatation of arterioles → peripheral **pooling of blood into the microcirculation** → **decreases** the **cardiac output** → produces **anoxic injury** to endothelial cell → favors development of **DIC** → widespread tissue hypoxia and **damage of vital organs**.

3. Irreversible phase:

- Without intervention, the shock eventually enters an irreversible stage.
- At this phase, **cellular and tissue injury is so severe** that even if the hemodynamic defects are corrected, **survival is not possible**.
- Widespread cell injury results in leakage of lysosomal enzymes, which aggravate the shock state.
- **Myocardial contractile function worsens** partly due to nitric oxide synthesis.
- If ischemic intestine allows microbes from the intestinal flora to enter into the circulation, it may lead to **superimposed bacteremic shock**.
- The patient develops **acute tubular necrosis** and results in death.

Stages of shock:

(1) Nonprogressive (2) Progressive (3) Irreversible.

Septic shock can initially cause cutaneous vasodilation, which produces warm skin.

Morphology (Table 5.10)

Q. Describe the morphological changes in various organs in shock.

Changes in Cardiogenic or Hypovolemic Shock:

These are mainly due to hypoxic injury. Morphological changes are particularly evident in adrenals, kidneys, lungs, brain, heart, and gastrointestinal tract.

- **Adrenal:**
 - **Lipid depletion in cortical cell:** It is due to conversion of the relatively inactive vacuolated cells to metabolically active cells. The active cells utilize stored lipids for the synthesis of steroids.
 - **Focal hemorrhage:** It occurs in the inner cortex of adrenal in **severe** shock.
 - **Massive hemorrhagic necrosis** of the entire adrenal gland is found in the Waterhouse-Friderichsen **syndrome**, which is **associated with severe meningococcal septicemia**.
 - Mention renal changes in shock
- **Kidney: Acute tubular necrosis** (acute renal failure) is a major complication of shock.

- **Gross:** Kidney is **enlarged, swollen, congested**, and the **cortex may appear pale**. Cut section shows blood pooling in the outer region of the medulla.
- **Microscopy:**
 - ♦ **Tubules:** Dilation of the proximal tubules and **focal necrosis of tubular epithelial cells**. Frequently, the tubular lumen may show **pigmented casts** formed due to leakage of hemoglobin or myoglobin.
 - ♦ **Interstitium:** It shows **edema** and **mononuclear cells** in the interstitium and within tubules.

Q. Write short note and lung changes in shock/diffuse alveolar change.

- **Lungs**
 - Lungs are relatively resistant to hypoxic injury and are usually not affected in pure hypovolemic shock.
 - However, when shock is due to bacterial sepsis or trauma, it shows **diffuse alveolar damage** which can lead to acute respiratory distress syndrome (ARDS) also known as shock lung.
 - **Gross:** The lung is **firm and congested**. Cut surface shows **oozing out of frothy fluid**.
 - **Microscopy:**
 - ♦ **Edema:** It first develops around peribronchial **interstitial** connective tissue and later in the **alveoli**.
 - ♦ **Necrosis:** Endothelial and alveolar epithelial cells undergo necrosis and leads to formation of **intravascular microthrombi**.
 - ♦ **Hyaline membrane:** It is usually seen lining the alveolar surface. It may also line alveolar ducts and terminal bronchioles.
- **Heart**
 - **Gross:** It shows **petechial hemorrhages** in the epicardium and endocardium.
 - **Microscopy:** **Necrosis** of the myocardium is seen which may range from minute focus to large areas of necrosis. Prominent **contraction bands** are seen by light microscopy.
- **Liver**
 - **Gross:** Liver is enlarged. Cut section shows a **mottled** (blotched) appearance due to marked pooling of blood in the centrilobular region.
 - **Microscopy:** The centrilobular region of the liver shows **congestion and necrosis**.
- **Brain:** Encephalopathy (ischemic or septic) and cortical necrosis.
- **Gastrointestinal tract:** Shock produces **diffuse gastrointestinal hemorrhage**. **Erosions of the gastric mucosa** and **superficial ischemic necrosis** in the intestine lead to gastrointestinal bleeding.

Shock lung: Diffuse alveolar damage.

Histological features of shock:

- ATN
- Depletion of lipids in adrenal cortex
- Pulmonary congestion
- Hepatic necrosis.

TABLE 5.10: Summary of main morphological features of shock

Organ	Changes
Adrenal	Lipid depletion in the cortical cells
Kidney	Acute tubular necrosis
Lungs	Relatively resistant to hypoxic injury. However, in septic shock shows diffuse alveolar damage (shock lung) with hyaline membrane
Heart	Coagulative necrosis and contraction band necrosis
Liver	Congestion and necrosis of centrilobular region of the liver
Brain	Encephalopathy (ischemic or septic) and cortical necrosis
Gastrointestinal tract	Diffuse gastrointestinal hemorrhage. Erosions of the gastric mucosa and superficial ischemic necrosis in the intestine.

Shock: Morphological changes mainly observed in adrenals, kidneys, lungs, brain, heart, and gastrointestinal tract.

Changes in Septic Shock

- Septic shock can lead to **DIC** which is characterized by **widespread** formation of fibrin-rich **microthrombi**, particularly in the brain, heart, lungs, kidney, adrenal glands, and gastrointestinal tract.
- The utilization of platelets and coagulation factors in DIC produces **bleeding manifestations**. It may show **petechial hemorrhages** on serosal surface and the skin.

Clinical Consequences

The clinical features of shock depend on the cause.

- **Hypovolemic and cardiogenic shock:** Usually present with features of hypotension and hypoperfusion. The features include **altered sensorium, cyanosis, oliguria, weak rapid pulse, tachypnea, and cool, clammy extremities**.
- **Septic shock:** The skin initially may be warm and flushed because of peripheral vasodilation.

The initial underlying cause that precipitated the shock may be life-threatening (e.g. myocardial infarct, severe hemorrhage, or sepsis). Later, the organ dysfunction involving **cardiac, cerebral, and pulmonary** function worsen the situation. The **electrolyte disturbances and metabolic acidosis** may further exacerbate the situation.

Patients who survive the initial complications may develop **renal insufficiency** characterized by a progressive decrease in urine output and severe fluid and electrolyte imbalances.

Cause of death in shock: Most commonly due to multiorgan failure.

Prognosis

The prognosis depends on the cause and duration of shock.

- Patients with hypovolemic shock may survive with appropriate management.
- Septic shock, or cardiogenic shock associated with massive myocardial infarction, usually have high mortality rate.

Diseases of the Immune System

The normal immune system is essential for protection against infection. Immune system is like a double-edged sword. Though it is protective in most of the situations, sometimes a hyperactive immune system may cause fatal diseases.

IMMUNITY

Definition: Immunity is **resistance** (defense mechanism) exhibited **by host against invasion by any foreign antigen**, including microorganisms.

Main physiological function of immune system is protection against infectious microbes.

Types: There are two types namely innate and adaptive immunity.

Innate (Natural/Native) Immunity

Immunity types: (1) Innate (2) Adaptive.

General Features

Innate immunity: Early and first line response to microbes.

- **First line of defense present by birth.**
- Provides **immediate initial protection** against an invading pathogen.
- **Does not depend on the prior contact** with foreign antigen or microbes.
- **Lacks specificity**, but **highly effective**. No memory, and no self/non-self recognition.

- **Triggers the adaptive immune response.**
- **No memory** is seen.

Major Components

Innate immunity components:

1. Physical barriers
2. Phagocytic cells, NK cells
3. Soluble plasma proteins (complements).

1. **Physical/anatomical barriers:** It includes epithelium lining skin, gastrointestinal and respiratory tracts which act as **mechanical barriers**, produce antimicrobial molecules such as defensins.
2. **Cells:**
 - **Phagocytic cells:** It consists of mainly **monocytes (macrophages in tissue) and neutrophils** in the blood. Phagocytic cells use several receptors to sense microbes and are called as “microbial sensors” (pattern recognition receptors).
 - **Pathogen associated molecular patterns (PAMPs):** Microbes have few highly conserved common molecular structures shared by entire classes of pathogens. These structures are called **pathogen associated molecular patterns (PAMPs)** and are essential for the infectivity of these pathogens.
 - **Pattern recognition receptors (PRRs):** Phagocytic cells involved in innate immunity recognize PAMP using a group of cellular receptors (microbial sensors) called pattern recognition receptors. Examples for PAMPs:

- ◆ **Toll-like receptors (TLRs).** These are transmembrane receptors and about 10 types of human TLRs have been identified. Each receptor recognizes a unique set of microbial patterns. For example, TLR2 recognizes various ligands (e.g. lipoteichoic acid) expressed by gram positive bacteria, TLR4 recognizes lipopolysaccharides (LPS) of gram negative bacteria.
- ◆ **Receptors for mannose residues**
- ◆ **NOD (nucleotide-oligomerization domain protein)-like receptors:** They are located in the cytoplasm and serve as intracellular sensors for microbial products.
- ◆ **Receptors for opsonins.**
- **Dendritic cells:** These cells function as antigen presenting cells to T-cells. They produce type I interferons (IFN) (e.g. IFN- α), which inhibit viral infection and replication.
- **Natural killer (NK) cells:** They provide defense against many viral infections and other intracellular pathogens (refer pages 116).

Toll-like receptor causes activation of NF- κ B and AP-1.

All gram negative bacteria (except leptospira) recognizes toll-like receptor-4 (TLR-4).

All gram positive bacteria and leptospira recognizes toll-like receptor-2 (TLR-2).

Natural killer cells: Attack cells which are not able to express MHC I.

3. Soluble molecules in the blood and tissues:

- **Complement system**
- Proteins that coat microbes and aid in phagocytosis, e.g. mannose-binding lectin and C-reactive protein.

Functions of Innate Immune Response

- **Inflammation** and destruction of invading microbe
- **Antiviral defense** is mediated by **dendritic cells and NK cells**.

Innate immunity: One of the manifestations is inflammatory response.

Adaptive Immunity

If the innate immune system fails to provide effective protection against invading microbes, the adaptive immune system is activated.

Adaptive immunity: Develops slowly but is more powerful and specialized than innate immunity.

General Features

Q. Write short note on cellular immunity.

Q. Write short note on humoral immunity.

- **Second line of defense** acquired during life
- **Capable of recognizing both microbial and nonmicrobial substances**
- **Takes more time to develop** and is **more powerful than innate immunity**
- **Long-lasting protection**
- Prior exposure to antigen is present
- Three **characteristic features** are: 1) **specificity**, 2) **diversity** and 3) **memory**.

Components

1. **Humoral immunity:** B lymphocytes and their soluble protein products called antibodies and helper T-cells.
2. **Cellular immunity:** T lymphocytes and their soluble products called cytokines.

Functions of Adaptive Immune Response

- **Antibodies:** Protection against extracellular microbes in the blood, mucosal secretions and tissues.
- **T lymphocytes:**
 - Defense against viruses, fungi and intracellular bacteria either by direct killing of infected cells by cytotoxic T lymphocytes or by activation of phagocytes to kill the ingested microbes.
 - Important immunoregulatory role, orchestrating and regulating the responses of other components of the immune system.

Humoral immunity: Mediated by antibodies secreted by B lymphocytes and are effective against extracellular microbes and their toxins.

Different types of adaptive immunity and their differences are shown in Table 6.1.

Both B and T lymphocytes express highly specific receptors for a wide variety of substances, called **antigens**.

TABLE 6.1: Differences between two types of adaptive immunity

Type	Mediator	Protection against
Humoral immunity	B lymphocytes which secrete antibodies	Extracellular microbes and their toxins
Cell-mediated (or cellular) immunity	T (thymus-derived) lymphocytes	Intracellular microbes

Terms "immune system" and "immune response" refer to adaptive immunity.

CELLS OF THE IMMUNE SYSTEM

Cells of immune responses (lymphocytes and other cells) migrate among lymphoid and other tissues and the vascular and lymphatic circulations.

CD45: Present in all leukocytes. Also known as leukocyte common antigen (LCA).

Naïve Lymphocytes

These are mature lymphocytes which have not encountered the antigen (immunologically inexperienced). After the lymphocytes are activated by recognition of antigens, they differentiate into:

- **Effector cells:** They perform the function of eliminating microbes.
- **Memory cells:** They live in a state of heightened awareness and are better able to combat the microbe in case it infects again.

Lymphocytes: Activated to proliferate and differentiate into (1) effector and (2) memory cells.

Memory T-cells can be identified by using the marker CD45RO.

T Lymphocytes

- **Development:** T (thymus-derived) lymphocytes develop from precursors in the thymus.
- **Distribution:** Mature T-cells are found in:
 - Peripheral blood where it constitute 60–70% of lymphocytes
 - T-cell zones of peripheral lymphoid organs namely paracortical region of lymph node and periarteriolar sheaths of spleen.
- **T-cell receptor:** T-cell recognizes a specific cell-bound antigen by means of an antigen specific T-cell receptor (TCR).
- **Markers:** Leukocyte cell surface molecules are named systematically by assigning them a 'cluster of differentiation' (CD) antigen number that helps in their identification.
 - **Primary T-cell associated CD molecules:** CD1, CD3, CD4, CD5 and CD8.
 - **CD3** is involved in signal transduction and is also known as **pan T-cell marker**. It is involved in T-cell activation.

- **Subsets of T lymphocytes:** Naïve T-cells can differentiate into two subtypes, namely **CD4** and **CD8**. Both subtypes serve as "coreceptors" in T-cell activation. They are called as coreceptors because they work with the antigen receptor in responses to antigen.

Q. Write short note on T helper cell.

- **CD4⁺ T-cell:** These subset of T-cells have CD4 molecule and are called as **helper T-cells**. They constitute about 60% of mature T-cells. The CD4 cells function as cytokine-secreting helper cells that help macrophages and B lymphocytes to combat infections. They are subcategorized as T_H1, T_H2 and T_H17 CD4⁺ T-cells.
- **CD8⁺ T-cell:** These subset of T-cells have CD8 molecule and are called as **cytotoxic/killer T-cells**. They constitute about 30% of T-cells. CD8⁺ T-cells function as cytotoxic (killer) T lymphocytes (CTLs) to destroy host cells harboring microbes and tumor cells.

CD4⁺ T-cells: Recognize and bind only to class II MHC molecules present on the antigen presenting cells (MHC-II restricted).

CD8⁺ T-cells: Recognize and bind only to class I MHC molecules present on the antigen presenting cells (MHC-I restricted).

CD4⁺ T-cell (helper cell): Master regulator of immune system.

When the antigen presenting cells (APCs) present antigen to T-cells, CD4⁺ T-cells recognize and bind only to class II MHC molecules and CD8⁺ T-cells bind only to class I MHC molecules.

Normal ratio between CD4⁺ T-cell and CD8⁺ T-cell is 2:1.

CD8⁺ ('cytotoxic') T lymphocytes: Recognize antigenic peptides in association with HLA class I molecules (HLA-A, HLA-B, HLA-C).

CD8⁺ T-cells: Kill infected cells directly through the production of pore-forming molecules such as perforin, or by triggering apoptosis of the target cell.

CD4⁺ helper T lymphocytes: Recognize peptides presented on HLA class II molecules (HLA-DR, HLA-DP and HLA-DQ).

CD4⁺ helper T-cells:

- Help B-cells to produce antibodies/immunoglobulin production
- Activate macrophages to destroy ingested microbes
- Stimulate leukocyte recruitment
- Regulate all immune responses to protein antigens.

Functions of CD4⁺ helper T-cell is mediated by cytokines.

Naive cells: Immunologically inexperienced mature lymphocytes that have not encountered the antigen for which they are specific.

Memory cells: Lymphocytes with heightened awareness and better able to combat the microbe (in case it returns).

Cytotoxic T-cells produce cytotoxic granules perforins and granzymes.

B Lymphocytes

- **Development:** B (bone marrow-derived) lymphocytes develop from precursors in the bone marrow.
- **Distribution:**
 - **Peripheral blood:** Mature B-cells constitute 10–20% of the circulating peripheral lymphocyte population.
 - **Peripheral lymphoid tissues:** Lymph nodes (cortex), spleen (white pulp), and mucosa-associated lymphoid tissues (pharyngeal tonsils and Peyer's patches of GIT).
- **B-cell receptor (BCR):** B-cells have receptors composed of IgM and IgD on their surface and has unique antigen specificity.
- **Functions of B-cells:** All the mature, naive B-cells express membrane-bound immunoglobulins (Ig) on their surface that functions as B-cell receptors (BCRs) for antigen. B-cells recognize antigen via these BCRs.
 - **Production of antibodies:** The primary function of B-cells is to **produce antibodies**. After stimulation by antigen and other signals, B-cells develop into **plasma**

cells. These cells secrete antibodies which are the mediators of humoral immunity. Salient features of various antibodies are presented in Table 6.2.

- **Antigen presenting cell:** B-cells also serve as APCs and are very efficient at antigen processing.
- **Markers:** B-cell markers include: CD 10 (CALLA), CD19, CD20, CD21 (EBV receptor), CD23, CD79a.

B-cells also express several receptors. Type 2 complement receptor (CR2, or CD21) is the receptor for the Epstein-Barr virus (EBV), and hence EBV infects B-cells.

CD19 is a pan B-cell marker and involved in signal transduction.

CD3 is a pan T-cell marker and involved in T-cell activation.

Dendritic Cells

As the name suggests these cells have numerous fine cytoplasmic processes that resemble dendrites. These are important antigen presenting cells in the body and can be functionally of the following types:

- **Interdigitating dendritic cells (IDC):** They are the **most important APCs** for initiating primary T-cell responses against protein antigens.
 - **Location:** (1) **Common location is below the epithelial lining: Immature dendritic cells within the epidermis are known as Langerhans cells.** (2) **Interstitial of all tissues.**

TABLE 6.2: Salient features of antibodies (immunoglobulins)

Features	IgM (millionaire's antibody)	IgG (subtypes: IgG1, IgG2, IgG3, IgG4)	IgA	IgE (reaginic/homocytotrophic antibody)	IgD
Approx % of total Ig	5%	80% (maximum)	15%	Trace	Trace
Molecular weight	900,000 (maximum)	150,000	150,000 to 300,000	190,000	180,000
Type of heavy chain	μ	γ	α	ϵ	δ
Structure	Pentamer (maximum size)	Monomer	Dimer (in glandular secretions), monomer (in serum)	Monomer	Monomer
Complement activation	Yes (classical pathway)	Yes (classical pathway)	Activates alternate complement pathway	No	No
Transport across placenta	No	Yes	No	No	No
Half-life (days)	5	21	6	2	3
Main function	Primary immune response	Secondary immune response Functions as B-cell receptor	Mucosal immunity Highly effective at neutralizing toxins	Allergic diseases, defense against parasite infection and anaphylactic reaction	Unknown

- **Follicular dendritic cell:**
 - **Location:** It is present in the germinal centers of lymphoid follicles in the spleen and lymph nodes (hence named as follicular dendritic cell).

Dendritic cells: Most efficient APCs which are located in epithelia and most tissues.

Langerhans cells are dendritic cells in the epidermis.

Follicular dendritic cell acts as reservoir for HIV in AIDS.

Macrophages

Q. Write short note on macrophage and its function.

- Macrophages are a part of the mononuclear phagocyte system.
- **Role in adaptive immune responses:**
 - **Processing of antigen:** Macrophages process the antigens present in the phagocytosed microbes and protein antigens. After processing, the antigen is presented to T-cells and thus, they function as APCs in T-cell activation.
- **Effector cell in immunity:**
 - **Cell-mediated immunity:** Macrophages are main effector cells in certain types of cell-mediated immunity, the reaction that serves to eliminate intracellular microbes. In this type of response, T-cells activate macrophages and increase their capability to kill ingested microbes.
 - **Humoral immunity:** Macrophages also participate in the effector phase of humoral immunity. Macrophages get activated by $\text{INF-}\gamma$.
 - **Phagocytosis:** Macrophages efficiently phagocytose and destroy microbes which are opsonized (coated) by IgG or C3b through their respective receptors.

Macrophage associated markers: CD13, CD14, CD15 and CD33.

Antigen-presenting cells:

1. Macrophages (wide distribution)
2. Langerhans cells (in skin)
3. Dendritic cells (in the mucosa, lymph and blood).

Natural Killer Cells

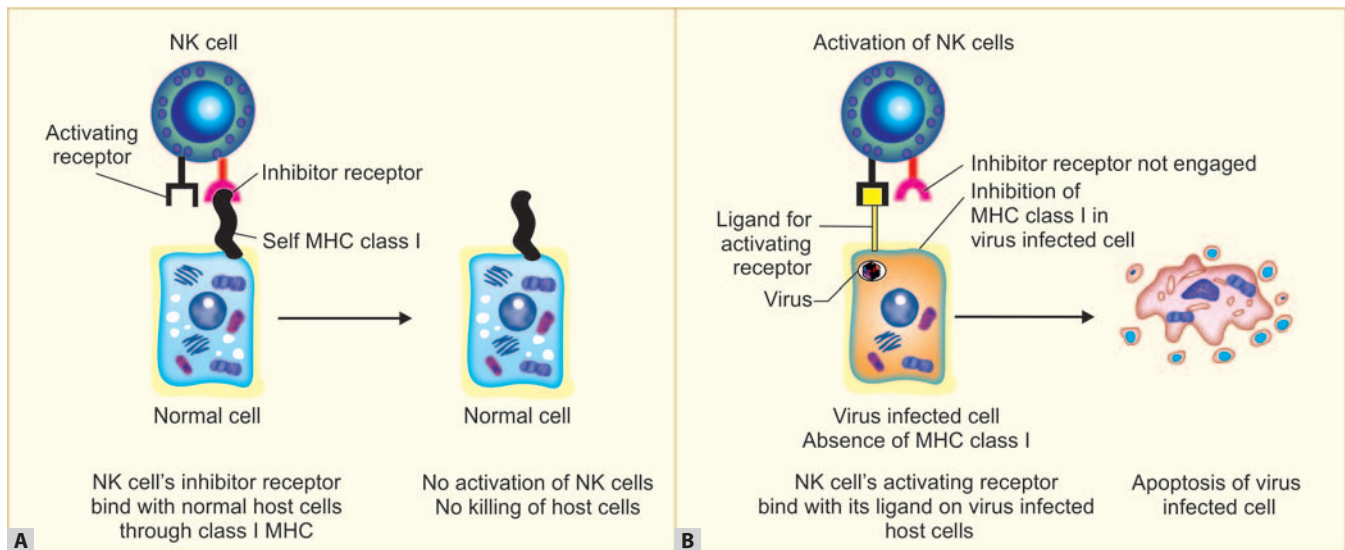
Q. Write short note on natural killer cell.

- Non-phagocytic **large** (little larger than small lymphocytes) **granular** (numerous cytoplasmic **azurophilic granules**) lymphocytes.

- **Markers:** They do not bear the markers for T- or B-cells. Two cell surface molecules, **CD16** and **CD56**, are commonly used to identify them.
- Comprise about 5–15% of **human peripheral lymphoid cells**.

Function

- Natural killer (NK) cells provide **defense against many viral infections** and other **intracellular pathogens** and also **has antitumor activity**, causing lysis of cells with which they react. Killing of the cells is performed without prior exposure to or activation by these microbes or tumors. Because of this ability, NK cells act as an early line of defense against viral infections and few tumors. They recognize abnormal cells in two ways:
 - **Antibody-dependent cellular cytotoxicity (ADCC):** NK cells bear (CD16) immunoglobulin receptors (F_cR) and bind antibody-coated targets leading to lysis of these cells. This phenomenon is called as antibody-dependent cell-mediated cytotoxicity.
 - **Perforin-granzymes system** (Figs 6.1 and 6.13): NK cells have a variety of **surface receptors for MHC** (major histocompatibility complex) **class I**. These receptors can either be having **inhibitory or activating functions**. The function of NK cells is regulated by a balance between signals from these activating and inhibitory receptors (Fig. 6.1).
 - ♦ **Inhibitory receptors:** MHC class I molecules are normally expressed on healthy/normal host cells. NK cell inhibitory receptors recognize self-class I MHC molecules, which are expressed on all normal healthy host cells (MHC class I positive). They prevent NK cells from killing normal host cells by inhibiting the death pathway.
 - ♦ **Activating receptors:** If the target cell with which NK cells interact, do not have MHC molecules on their surface, there is no binding of MHC receptor of NK cells. The downregulation of class I MHC molecules (leading to absence of MHC molecules) may occur in cells due to various kinds of stress such as infection by viruses and DNA damage as in tumor. These activating receptors make holes in the target cell membrane by secreting perforins. Granzymes secreted by NK cells are injected through these pores and cause apoptosis of target cell (Fig. 6.13). NK cells kill cells that are infected by some microbes or cells that are damaged beyond repair.



Figs 6.1A and B: Function of natural killer (NK) cells: (A) Normal host cells express self-class I MHC molecules, which are recognized by inhibitory receptors of NK cells that binds them and prevent from killing normal cells; (B) In infected and stressed cells, class I MHC expression is reduced so that the inhibitory receptors of NK cells are not engaged. This results in activation of NK cells and killing of infected cells/stressed cells

Effector cells of immune system:

- NK cells
- Plasma cells
- CD4⁺ T helper cells
- CD8⁺ CTLs.

Cell lysis by NK cells is unique:

1. Not mediated by immune response
2. MHC unrestricted
3. Does not involve an antigen-antibody interaction.

Ability of NK cells to kill target cells is inversely related to target cell expression of MHC class I molecules.

Hyporesponsiveness of NK cells found in Chediak-Higashi syndrome.

CYTOKINES

- Immune responses involve multiple interactions among many cells. These include lymphocytes, dendritic cells, macrophages, other inflammatory cells (e.g. neutrophils), and endothelial cells.
- Some of these interactions are cell-to-cell contact. However, many interactions and effector functions of leukocytes are mediated by **short-acting soluble proteins** called **cytokines**. These cytokines represent the **messenger molecules of the immune system** and mediate communications between leukocytes and are called **interleukins**.

Cytokines: Messenger molecules of the immune system.

Classification

Most of the cytokines have many effects and can be classified depending on their functions.

Cytokines of Innate Immunity

- These cytokines are produced rapidly in response to microbes and other stimuli
- Mainly secreted by macrophages, dendritic cells and NK cells
- Mediate inflammation and antiviral defense
- These cytokines include TNF, IL-1, IL-12, type I IFNs, IFN- γ and chemokines.

Cytokines of Adaptive Immune Responses

- These cytokines are produced mainly by CD4⁺ T lymphocytes in response to antigen and other signals
- They promote lymphocyte proliferation and differentiation and activate effector cells
- This category include IL-2, IL-4, IL-5, IL-17, and IFN- γ .

Colony-Stimulating Factors

- These cytokines stimulate hematopoiesis and are assayed by their ability to stimulate formation of blood cell colonies from bone marrow progenitors.
- They increase leukocyte numbers during immune and inflammatory responses.

HYPERSENSITIVITY REACTIONS

Immune response is usually a protective process but sometimes it may be injurious. Hypersensitivity means that the body responds to a particular antigens in an exaggerated fashion, where it does not happen in normal circumstances.

Definition: Hypersensitivity reaction is a **pathological, excessive and injurious immune response to antigen leading to tissue injury, disease** or sometimes death in a **sensitized individual**. The resulting diseases are named as hypersensitivity diseases.

Hypersensitivity reaction: Pathological, excessive and injurious immune response to antigen leading to tissue injury.

General Features of Hypersensitivity Disorders

- **Priming or sensitization:** It occurs in **individuals who had previous contact with the antigen** (allergen).
- **Nature of antigens:** It may be **exogenous or endogenous** origin.
 - **Exogenous antigens:** For example, antigens in dust, pollen, food, drugs, microbes, chemicals and few blood products.
 - **Endogenous antigens:** Self or autologous antigens → cause **autoimmune diseases**.
- **Genetic susceptibility:** Hypersensitivity diseases are usually associated with the inheritance of particular susceptibility genes (e.g. HLA genes).
- **Imbalance between control and effector mechanisms:** It produces damage to host tissues.
- **Mechanism of tissue injury:** Same as the effector mechanisms of defense against infectious pathogens.
 - However, these reactions are poorly controlled, excessive, or misdirected (e.g. against normally harmless environmental and self antigens).

Classification of Hypersensitivity Reactions (Table 6.3)

TABLE 6.3: Classification of hypersensitivity reaction according to the effector immune mechanism

Types	Effectors
1. Immediate hypersensitivity reaction (type I hypersensitivity)	Antibody molecules
2. Antibody-mediated disorders (type II hypersensitivity)	

Contd...

Contd...

3. Immune complex-mediated disorders (type III hypersensitivity)	
4. Cell-mediated immune disorders (type IV hypersensitivity)	Antigen-specific effector T-cells

Cell-mediated immunity: Mediated by T lymphocytes and protects against intracellular microbes.

TYPE I (IMMEDIATE) HYPERSENSITIVITY REACTIONS

Q. Write short note on type I hypersensitivity reactions.

Usually known as **allergic or atopic disorders** and the environmental antigens that elicit these reactions are known as **allergens**.

Definition: Type I hypersensitivity reaction is a type of immunological tissue reaction, which **occurs rapidly (within 5–10 minutes) after the interaction of antigen** (allergen) **with IgE antibodies bound to the mast cells in a sensitized person**.

Allergen: Antigen that evoke allergic response.

Immunoglobulin involved in type I hypersensitivity reaction: IgE.

Characteristics

- **Immediate** reaction occurring within minutes (5–10 minutes). Most are caused by excessive T_H2 responses.
- **Antibodies:** Mediated by IgE antibody.
- **Develops after the interaction of an antigen with IgE antibodies bound to mast cells.**
- **Genetic susceptibility:** Occurs in genetically susceptible individuals **previously sensitized** to the antigen.
- **Antigens (allergens):** Many allergens (e.g. house-dust mite, pollens, animal danders or moulds) in the environment are **harmless for majority of individuals**. Allergens elicit significant IgE reactions only in **genetically predisposed individuals**, who are said to be **atopic**.

Sequence of Events (Fig. 6.2)

Q. Write short note on anaphylactic shock.

During Initial Exposure to Antigen (Sensitization)

In a genetically susceptible individual, the following events occur:

1. **Exposure to sensitizing antigen:** Individuals are exposed to environmental allergens and may be introduced by: (1) **inhalation**, (2) **ingestion** or (3) **injection**.
 2. **Presentation of the antigen:** The **sensitizing antigen (allergen)** is **presented to T-cells**. However, T-cells do not recognize antigens by themselves but recognize when presented **by antigen presenting cells (APC)**, which capture the antigen.
- Type I hypersensitivity: Produced by environmental antigens (allergens) in a genetically susceptible individuals.
3. **Activation of T_H2 cells:** In genetically susceptible individual, antigens (allergens) activate **T_H2 subset of $CD4^+$ helper T-cells** → **secretes cytokines (e.g. IL-4, IL-5 and IL-13)**.
 4. **Production of IgE antibody:** IL-4 secreted by T_H2 cells stimulates B-cells to secrete cytotoxic IgE antibodies. IL-5 activates eosinophils and IL-13 stimulates epithelial cells to secrete mucus.
 5. **Sensitization of mast cells by IgE antibody:**
 - **Mast cells** are mainly concentrated near blood vessels and nerves and in subepithelial tissues (common sites of type I hypersensitivity).
 - Mast cells possess F_{ϵ} -epsilon ($F_{\epsilon}R1$) receptor, which have high affinity for IgE antibodies.
 - IgE antibodies produced by B-cells attach to the $F_{\epsilon}R1$ on the mast cells. These **IgE antibody bearing mast**

cells are sensitized to react if antigens binds to these antibodies.

- **Eosinophils** also express $F_{\epsilon}R1$ and are involved in IgE mediated defense against helminth infections.

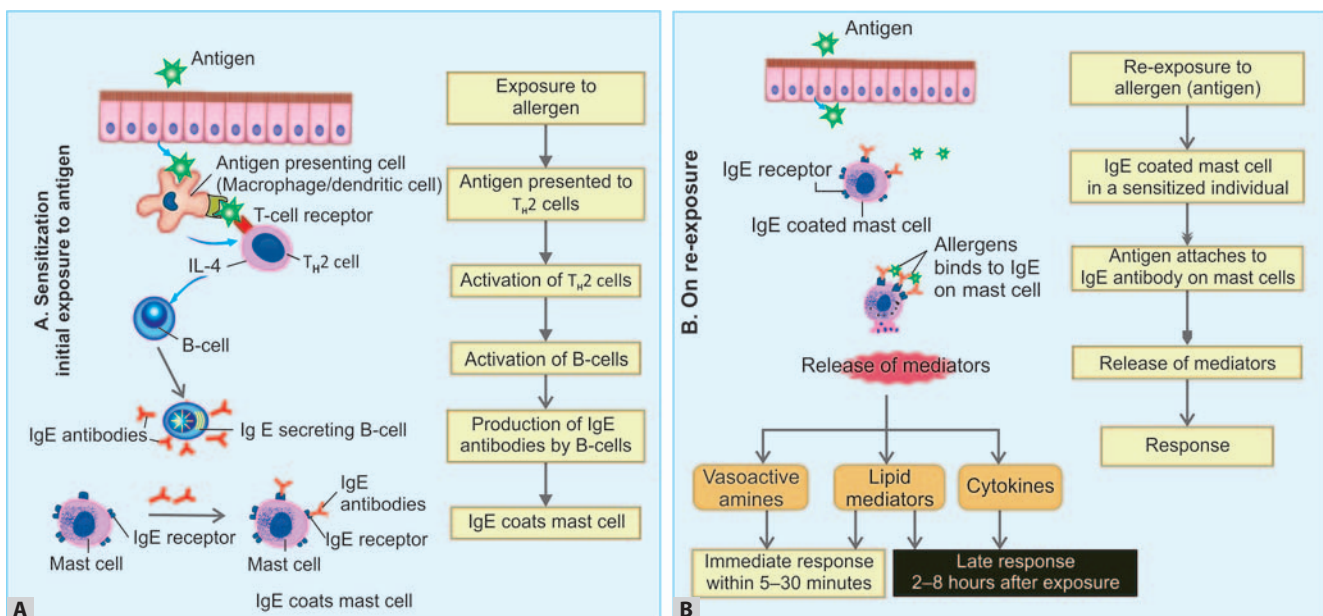
T_H2 cells: Play a central role in immediate hypersensitivity reactions.

Type I hypersensitivity: First exposure to allergens elicit a strong T_H2 response which stimulates production of IgE by B-cells → IgE attaches to mast cells.

During Subsequent Exposure to Antigen

In sensitized individual (the mast cell has attached IgE antibodies), during subsequent re-exposure to the specific allergen, following events occur:

- **Mast cell activation:** The **antigen (allergen)** binds to more than one **IgE antibody molecules on mast cells** → generate signals → **causes mast cell degranulation** → **secretion of preformed (primary) mediators** that are stored in the granules.
- **Two phases:** IgE triggered reactions can be divided into two phases:
 - **Immediate response:**
 - ◆ Develops within 5–30 minutes after exposure to an allergen and subside in 60 minutes.
 - ◆ Characterized by vasodilation, vascular leakage, and smooth muscle spasm or glandular secretions.



Figs 6.2A and B: Sequence of events in type I hypersensitivity. (A) It is initiated by the exposure to an allergen, which stimulates T_H2 responses and IgE production, in genetically susceptible individuals. IgE binds to mast cells; (B) On re-exposure to the allergen, antigen binds to IgE on the mast cells and activates it to secrete the mediators. These mediators produce the manifestations of type I hypersensitivity

– **Late-phase reaction:**

- ◆ Develops in 2–8 hours after the exposure to antigen which may **last for several days**.
- ◆ Characterized by **infiltration of tissues with eosinophils, neutrophils, basophils, monocytes, and T_H2 cells**. It also shows mucosal **epithelial cell damage**.

Type I hypersensitivity: On re-exposure antigens cross-link IgE and stimulate mast cell to secrete mediators.

Type I hypersensitivity reaction: Release of mediators occur in two phases:

- Immediate response
- Late-phase reaction.

Mediators of Type I Hypersensitivity Reactions (Fig. 6.3)

1. **Preformed mediators (primary mediators):** They are stored in mast cell granules. Their biological effects start immediately following their release. These include:

- **Vasoactive amines:** Most important being **histamine**, which causes:
 - **Vasodilatation**
 - **Increased vascular permeability**
 - **Smooth muscle contraction**
 - **Increased secretion of mucus** by nasal, bronchial and gastric glands.

- **Enzymes:** It includes neutral proteases (chymase, tryptase) and several acid hydrolases. These enzymes **cause tissue damage** and generate kinins and activates components of complement (e.g. C3a) by acting on their precursor proteins.
- **Proteoglycans:** It includes heparin (anticoagulant), and chondroitin sulfate.
- Neutrophil and eosinophil chemotactic factors (NCF and ECF).

2. **Secondary (newly synthesized) mediators:**

- **Lipid mediators:** They are synthesized and secreted by mast cells, includes leukotrienes and prostaglandins.
 - **Leukotrienes C₄ and D₄** (previously known as the slow-reacting substances of anaphylaxis -SRS-A) These are the most powerful (several thousand times than histamine) and cause **increased vascular permeability** and **bronchial smooth muscle contraction**.
 - **Leukotriene B₄** is **chemotactic** for neutrophils, eosinophils and monocytes.
 - **Prostaglandin D₂**: It causes bronchospasm and increased mucus secretion.
- **Cytokines:** Mast cells can produce many cytokines, which may be involved in immediate hypersensitivity reactions. The cytokines include:
 - TNF, IL-1 and chemokines promote leukocyte recruitment (during the late-phase reaction).
 - IL-4 and IL-5 amplifies the T_H2 response and IL-13 stimulates mucus secretion by epithelial cells.

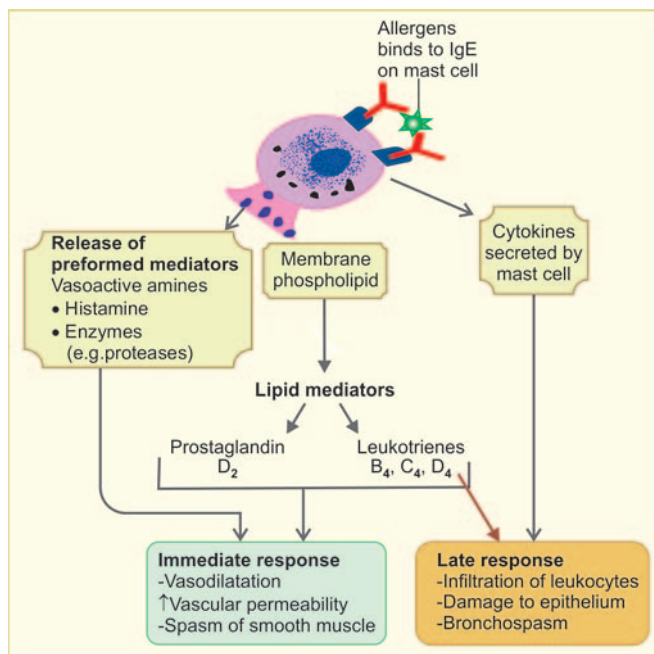


Fig. 6.3: Mast cell mediators involved in type I hypersensitivity

Type I hypersensitivity reaction commonly referred as allergy.

Type I hypersensitivity: Principal mediators involved are:

1. Histamine
2. Enzymes (e.g. proteases)
3. Prostaglandins
4. Leukotrienes
5. Cytokines.

IL-4: Responsible for secretion of IgE from the B-cells.

IL-5: Most potent eosinophil-activating cytokine.

Eosinophils in Type I Hypersensitivity Reaction

- Eosinophils are important effector cells of tissue injury during late-phase reaction.
- They are recruited by chemokines such as eotaxin and others produced by epithelial cells, T_H2 cells and mast cells.
- Eosinophils products:
 - Major basic protein and eosinophil cationic protein → damage the epithelial cells.
 - Leukotriene C₄ and platelet-activating factor (PAF) → promote inflammation.

Clinical Manifestations

Systemic Anaphylaxis

- Acute, potentially fatal form and known as anaphylaxis (ana = without, phylaxis = protection).
- Usually follows injection of an antigen into a sensitized individual.
- May cause shock and death
- Causes: It develops:
 - After administration of foreign proteins (e.g. antisera), drugs (e.g. penicillin), hormones and enzymes
 - Following exposure to food allergens (e.g. peanuts, shellfish) or insect toxins (e.g. bee venom)
- Dose: Systemic anaphylaxis may be triggered by extremely small doses of antigen.
- Clinical features:
 - Itching, hives and skin erythema appear within minutes after exposure
 - Followed by difficulty in breathing and respiratory distress due to contraction of respiratory bronchioles
 - Laryngeal edema results in hoarseness and laryngeal obstruction, which further aggravates respiratory difficulty
 - Vomiting, abdominal cramps, diarrhea may follow
 - May lead to shock and death within an hour.

Local Reactions

- Recurrent and nonfatal
 - Site of local reaction depends on the portal of entry of the allergen
- Causes: Develop against common environmental allergens, such as pollen, animal dander, house dust, and food.

Type I hypersensitivity: It may manifest as systemic fatal anaphylaxis or more commonly as local reactions.

Bee sting reaction is mediated by IgE (type I hypersensitivity).

Atopy

- It refers to a familial predisposition to produce an **exaggerated localized immediate hypersensitivity (IgE mediated) reactions to inhaled and ingested environmental substances** (allergens) that are otherwise harmless.
- Atopic individuals tend to have **higher serum IgE levels, and more IL-4 producing T_H2 cells**.
- A positive family history of allergy is found in 50% of atopic individuals.

Examples of type I hypersensitivity reactions are listed in Table 6.4.

TABLE 6.4: Examples of type I hypersensitivity reactions

Localized type I hypersensitivity	Systemic type I hypersensitivity
<ul style="list-style-type: none"> Bronchial asthma (extrinsic) Hay fever/allergic rhinitis Allergic conjunctivitis Urticaria Atopic dermatitis /eczema Angioedema Allergic gastroenteritis (food allergy) 	Anaphylaxis due to: <ul style="list-style-type: none"> Antibiotics: Most commonly penicillin Bee stings Insect bite Foreign proteins (e.g. antisera), Foods (peanuts, fish and shellfish) Food additives

Diagnosis of Type I Hypersensitivity

- Typical clinical history and examination
- Skin-prick testing
- Measuring specific IgE in the serum.

Anaphylactoid Reactions

- Non-IgE mediated** that is indistinguishable from anaphylactic reactions
- Most non-IgE-dependent foreign agents do not require antigen processing (sensitization) and can elicit a mast cell activation response on **first antigen exposure** itself
- Short lived** because it involves only degranulation of mast cells and not cytokine synthesis.

ANTIBODY-MEDIATED (TYPE II) HYPERSENSITIVITY REACTIONS

Q. Write short note on type II hypersensitivity reactions.

Definition: Type II hypersensitivity disorders are caused by antibodies (IgG/IgM), which react with **target antigens on the surface of cells or fixed in the extracellular matrix**.

Type II hypersensitivity: Caused by antibodies (IgG/IgM), that react with antigens on the surface of cells or fixed in the extracellular matrix.

Characteristics

Antibodies: IgG (usually) and IgM (rarely) type of antibodies mediate type II reactions.

Antigen: It may be endogenous or exogenous

- **Endogenous antigens:** It may be normal molecules intrinsic to the cell membrane or extracellular matrix (e.g. autoimmune diseases).
- **Exogenous antigens:** These antigens may get adsorbed on a cell surface or extracellular matrix → may cause altered surface antigen (e.g. drug metabolite).

Antibody mediated (type II) hypersensitivity reaction:

1. Transfusion reactions
2. Hemolytic disease of newborn
3. Autoimmune hemolytic anemia.

Mechanism of Injury

Mechanism of type II hypersensitivity:

1. Complement dependent
 2. Complement independent.
- In type II hypersensitivity reactions, target antigens on cell surface or matrix antigens undergo chemical modification.
 - B-cells produce IgG antibodies against this modified antigen and IgG antibodies bind to these modified cells.

Mechanism of tissue injury can be broadly divided into: (1) complement dependent and (2) antibody-dependent.

Complement Dependent Reactions

Complement dependent reactions:

1. Opsonization and phagocytosis
 2. Lysis by MAC
 3. Complement and F_c receptor mediated inflammation.
1. **Opsonization and phagocytosis** (Fig. 6.4A): Complement injure the target cells by promoting their phagocytosis.
 - **Production of antibodies:** Antigen may be intrinsic to target cells (e.g. RBC or platelets) or exogenous antigen adsorbed to its cell surface. B-cells produce IgG antibodies (e.g. autoantibodies) against target antigens.
 - **Activation of complement:** Antigen antibody complexes are formed on the surfaces of the target

cells → may activate the complement system by the classical pathway.

- **Opsonization: Complement components such as C3b, which acts as opsonins** and gets deposited on the surfaces of the target cells.
- **Phagocytosis:** Opsonized cells are recognized by phagocytes through F_c and C3b receptors on its surface → results in **phagocytosis of the opsonized cells** → **destruction of cells by phagocytes** (e.g. macrophages in spleen).

Examples:

- **Autoimmune hemolytic anemia:** Target antigen is RBC membrane protein (Rh or I antigen).
- **Autoimmune thrombocytopenia purpura:** Target antigen is $GpIIb/IIIa$ of platelets
- **Drug-induced hemolytic anemia.**

2. **Lysis of target cells through membrane attack complex** (Fig. 6.4B): Complement causes lysis of target cells by generating membrane attack complex (C5-9).

- **Complement activation on cells also generates membrane attack complex (MAC).**
- MAC disrupts membrane integrity and causes **lysis of the cells.**
- **Example:** (1) **Transfusion reactions** in which the cells from an incompatible donor react with and are opsonized by preformed antibody in the recipient. (2) Hemolytic disease of newborn.

3. **Tissue injury by complement and F_c receptor mediated inflammation** (Fig. 6.5): Complement induces inflammation and causes injury to target cells.

- **Antibodies against matrix components in fixed tissue antigens**, such as basement membranes and extracellular matrix may **activate complement system by classical pathway.**
- Complement components may **cause injury due to inflammation.** This may be due to 1) chemotactic agents (mainly C5a) produced at the site of deposition of antibody and 2) anaphylatoxins (C3a and C5a), which increase vascular permeability.
- Activated **inflammatory cells** (leukocyte) **release lysosomal enzymes and reactive oxygen species** which **damage tissues.**
- Inflammation may also be induced by antibody binding to F_c receptors of leukocytes.
- **Example: Goodpasture syndrome** in which anti-glomerular basement membrane antibody binds to a glomerular basement membrane antigen and

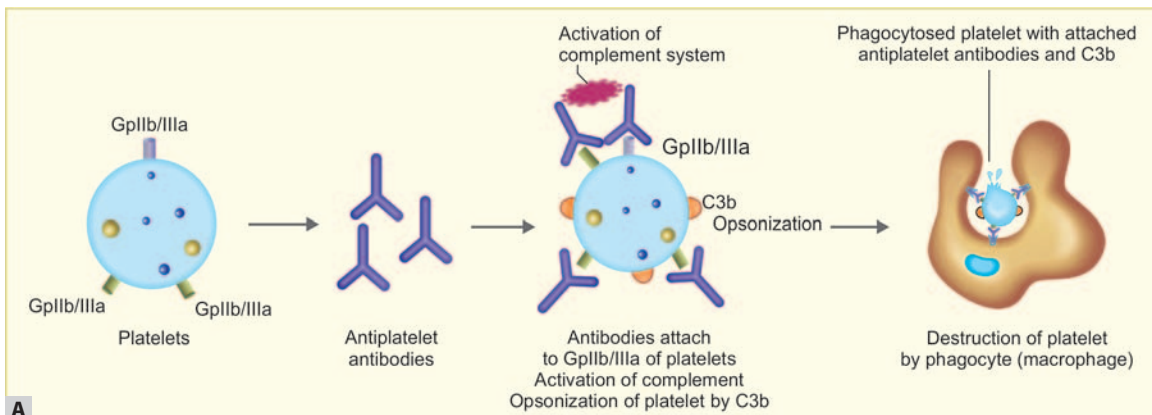


Fig. 6.4A: Type II hypersensitivity reaction: Complement dependent opsonization and phagocytosis. The antibody binds to antigens on the target cell. Activation of complements produces opsonin C3b. Opsonization of target cells by antibodies and complement leads to ingestion by phagocytes (phagocytosis of target cell) via either F_c or C3b receptors

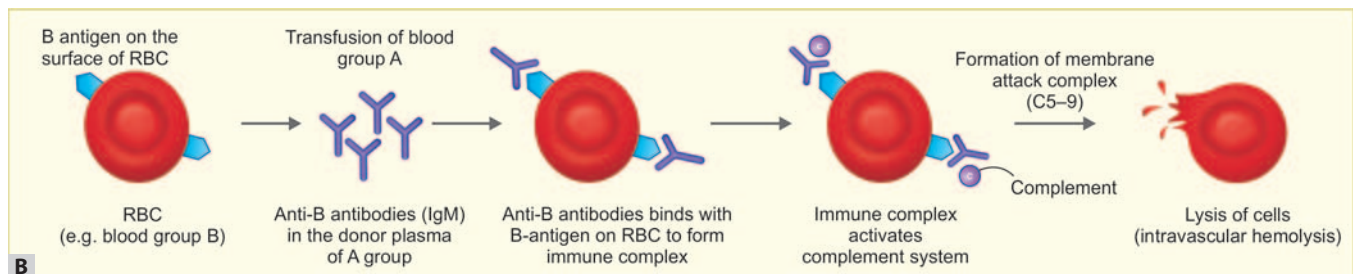


Fig. 6.4B: Type II hypersensitivity reaction: cell lysis through MAC. Binding of IgG or IgM antibody to an antigen promotes complement fixation. Activation of complement leads to formation of membrane attack complex (MAC) which causes cell lysis. Example—transfusion of A group blood to individual with B group

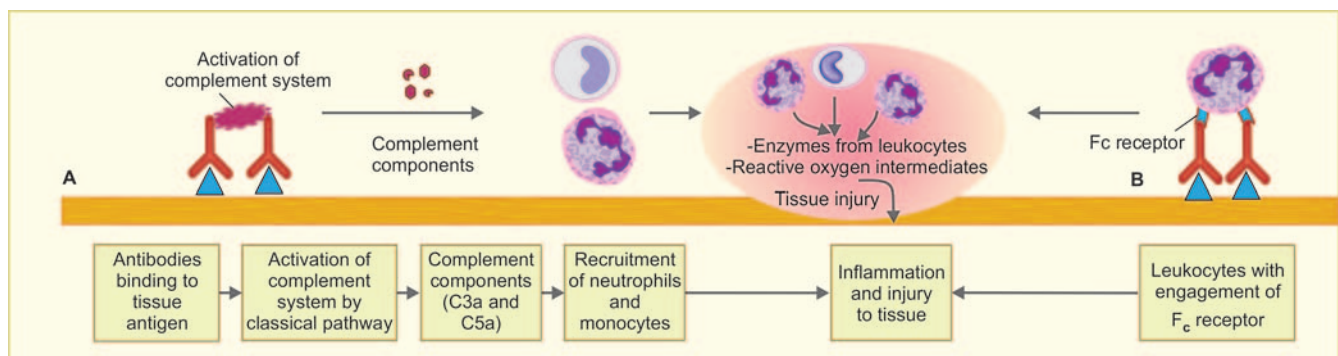


Fig. 6.5: Type II-hypersensitivity reaction—complement and F_c receptor mediated inflammation: (A) Antibody binds to a surface antigen, activates the complement system and leads to the recruitment of tissue-damaging inflammatory cells. Several complement-derived peptides (e.g. C5a) are potent chemotactic factors; (B) Inflammation may also be induced by antibody binding to F_c receptors of leukocytes

activates the complement system. The recruitment of inflammatory cells damages the basement membrane.

Type II hypersensitivity:

- Antibodies can coat (opsonize) cells with or without complement and target these cells for phagocytosis by macrophages.
- Macrophages express F_c receptor and receptor for complement.

Antibody-Dependent (Complement Independent) Cellular Dysfunction

It is characterized by deposition of antibodies against target cell surface receptors, which may impair or dysregulate function of the target cell without causing cell injury or inflammation. Examples:

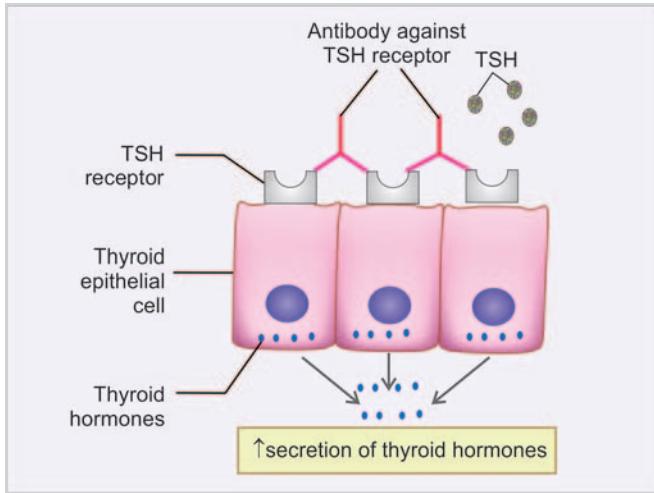


Fig. 6.6: Type II hypersensitivity reaction: Antibody-mediated stimulation of cell function. Autoantibodies bind against the thyroid-stimulating hormone (TSH) receptor and activate thyroid cells to produce excessive production of hormones and causing hyperthyroidism in Graves' disease

- **Antibody-mediated stimulation of cell function:** In **Graves' disease**, antibodies against the thyroid-stimulating hormone receptor on thyroid epithelial cells stimulate the cells. This results in hyperthyroidism (Fig. 6.6).
- **Antibody-mediated inhibition of cell function:** In **myasthenia gravis** (Fig. 6.7), antibodies directed against acetylcholine receptors in the motor end plates of skeletal muscles block neuromuscular transmission. This causes muscle weakness.

Type II hypersensitivity: Antibody-dependent cellular dysfunction to:

- Stimulation of cell function or
- Inhibition of cell function.

Mechanism of type II hypersensitivity reactions are summarized in Figure 6.8.

Examples of type II hypersensitivity diseases are presented in Table 6.5.

IMMUNE COMPLEX-MEDIATED (TYPE III) HYPERSENSITIVITY REACTIONS

Q. Write short note on type III hypersensitivity reactions.

Type III hypersensitivity reactions: Immune complexes activate complement and acute inflammation causing tissue damage.

Definition: Type III hypersensitivity reactions are characterized by formation of immune (antigen and antibody)

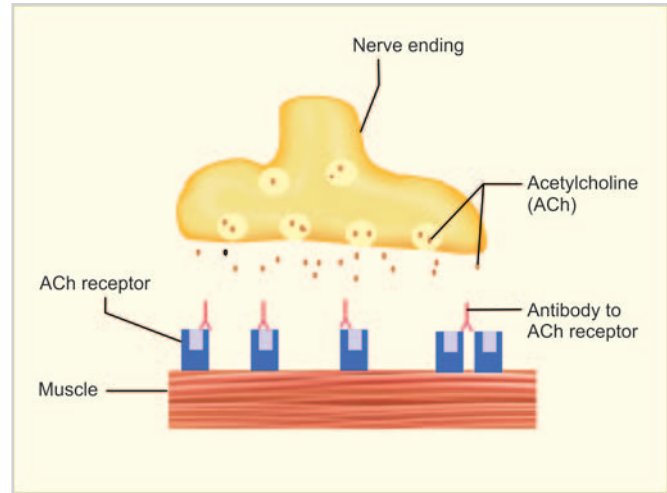


Fig. 6.7: Type II hypersensitivity reaction: Antibody-mediated inhibition of cell function. Anti-receptor antibodies may inhibit/disturb the normal function of receptors. Example—autoantibodies to the acetylcholine (ACh) receptor on skeletal muscle cells in myasthenia gravis produce disease by blocking neuromuscular transmission and causing progressive muscle weakness

complexes in the circulation and may get deposited in blood vessels, leading to complement activation and acute inflammation. The inflammatory cells recruited (neutrophils and monocytes) release lysosomal enzymes → generate toxic free radicals → cause tissue damage.

Characteristics

Antibodies: Complement-fixing antibodies namely IgG, IgM and occasionally IgA.

Antigen:

- **Exogenous:** Various foreign proteins, e.g. foreign serum protein injected (e.g. diphtheria antitoxin, horse anti-thymocyte globulin) or produced by an infectious microbe.
- **Endogenous:** Antibody against self-components (autoimmunity), e.g. nucleoproteins.

Sites of Antigen-antibody Formation

- **Circulating immune complexes:** They are formed within the circulation.
- **In situ immune complex:** They formed at extravascular sites where antigen might have been previously planted.

Type III hypersensitivity: Reaction differs from type II in that the antigens are not attached to the cell but are free in the circulation.

Sites of Immune Complex Deposition

- **Systemic:** Circulating immune complexes may be deposited in many organs.

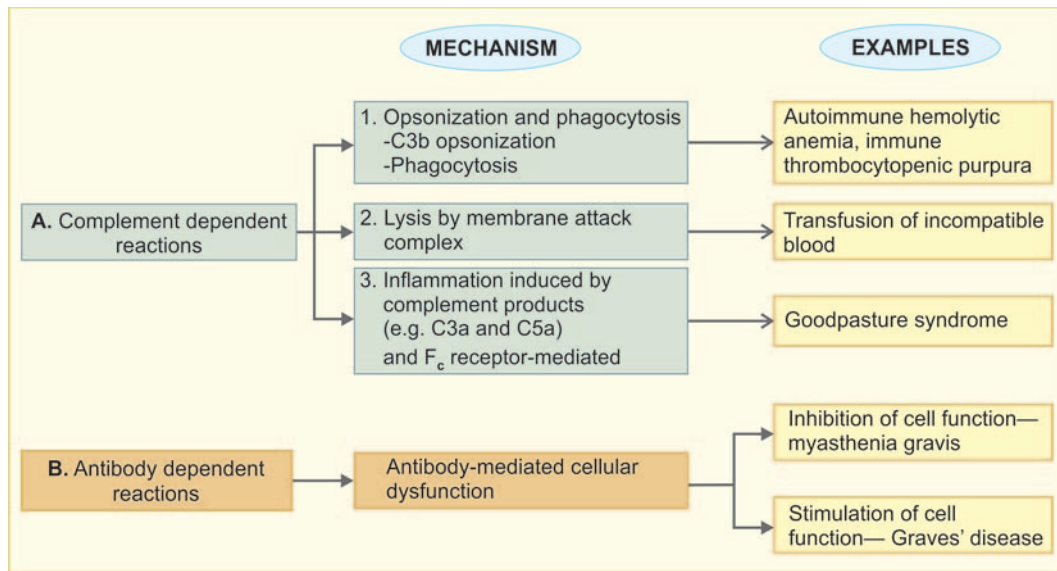


Fig. 6.8: Summary of mechanism of type II hypersensitivity reactions

TABLE 6.5: Examples of type II hypersensitivity (antibody-mediated) diseases

Disease	Target antigen	Mechanism of disease
A. Complement Dependent Reactions		
1. Opsonization and phagocytosis (IgG-mediated)		
Autoimmune hemolytic anemia	Cell-surface antigens (Rh blood group antigens, I antigen)	Opsonization and phagocytosis of RBCs
Autoimmune thrombocytopenic purpura	Platelet membrane glycoprotein IIb/IIIa integrin	Opsonization and phagocytosis of platelets
2. Complement-mediated lysis by membrane attack complex (IgM-mediated)		
Transfusion reactions	The cells from an incompatible donor react with and are opsonized by preformed antibody in the recipient	Complement activation and lysis by membrane attack complex
3. Complement and F_c receptor-mediated inflammation (IgG-mediated)		
Goodpasture syndrome	Antibody against matrix antigens (basement membrane noncollagenous protein of kidney glomeruli and lung alveoli)	Complement- and F _c receptor-mediated inflammation
B. Antibody-mediated (Complement Independent) Cellular Dysfunction		
Graves' disease (hyperthyroidism)	Antibody against receptors: Thyroid-stimulating hormone (TSH) receptor (agonistic antibodies)	Antibody-mediated stimulation of TSH receptors
Myasthenia gravis	Antibody against receptors: Acetylcholine receptor (antagonistic antibodies)	Antibody inhibits acetylcholine binding to receptors

- **Localized:** Immune complexes may be **deposited or formed in particular organs/tissues:** Kidney (glomerulonephritis), joints (arthritis), small blood vessels of the skin.

Mere presence of immune complexes does not indicate type III hypersensitivity.

Cause of Tissue Damage

- **Activation of complement**
- **Inflammation at the sites of deposition.**

Examples of immune complex disorders are listed in Table 6.6.

Q. Write short note on serum sickness.

Q. Write short note on Arthus reaction.

Type III hypersensitivity reactions: Autoimmune diseases such as SLE and many types of glomerulonephritis.

Systemic Immune Complex Disease—Acute Serum Sickness

This was a frequent sequela to the administration of large amounts of foreign serum (e.g. serum from immunized horses used for protection against diphtheria). Nowadays it is infrequent.

Pathogenesis (Fig. 6.9)

Divided into three phases:

1. **Formation of immune complexes:**
 - **Introduction of protein antigen:** It initiates an immune response.
 - **Formation of antibody:** It usually forms a week (7 to 12 days) after the injection of the foreign protein and are secreted into the blood.
 - **Formation of immune complexes:** They are formed in the circulation when antibodies react with the antigen.
2. **Deposition of immune complexes:**
 - **Immune complexes of medium size** and with **slight antigen excess** are the **most pathogenic**.
 - **Sites of deposition:**
 - **Blood vessels:** It causes vasculitis.
 - **Renal glomeruli:** It causes glomerulonephritis.
 - **Joints:** It causes arthritis.
3. **Inflammatory reaction and tissue injury:** Mechanism of tissue injury include:
 - **Inflammatory reaction:** Immune complexes in the tissue **activates complement**, the products (e.g. chemotactic C5a) of which causes **chemotactic recruitment of acute inflammatory cells** (neutrophils and monocytes) to the site.

TABLE 6.6: Examples of immune complex-mediated diseases

Disease	Antigen	Manifestations
Exogenous antigen		
Poststreptococcal glomerulonephritis	Streptococcal cell wall antigen(s)	Glomerulonephritis
Serum sickness	Various proteins, e.g. foreign serum protein (horse anti-thymocyte globulin)	Arthritis, vasculitis, nephritis
Arthus reaction	Various foreign proteins	Cutaneous vasculitis
Endogenous antigen		
Systemic lupus erythematosus (SLE)	Nuclear antigens	Glomerulonephritis, skin lesions, arthritis, others

- **Tissue damage:** Activated inflammatory cells (leukocyte) **release lysosomal enzymes, arachidonic acid products and reactive oxygen species** → which produce **tissue damage**.

Clinical features: Fever, urticaria, joint pains (arthralgias), lymph node enlargement and proteinuria appear during this phase.

Type III hypersensitivity reactions: Immune complexes are deposited in the tissues, activate complement system which leads to localized inflammatory response with recruitment of neutrophils and monocytes.

Type III hypersensitivity: Immune complexes of **medium size** and with **slight antigen excess** are pathogenic.

Type III hypersensitivity: During the active phase, activation of complement system leads to a decrease level of C3 in the serum and can be used to monitor disease activity.

MORPHOLOGY

General Features

- **Acute necrotizing vasculitis:** It is the **main feature** and is characterized by **necrosis of the vessel wall and intense neutrophilic infiltration**.
- **Fibrinoid necrosis:** It consists of necrotic tissue, immune complexes deposits, complement and plasma protein. It produces a **smudgy eosinophilic appearance** at the site of deposit and obscures the cellular detail.

Kidney

- **Immunofluorescence microscopy:** It appears as **granular lumpy deposits** of immunoglobulin and complement.
- **Electron microscopy:** It appears as **electron-dense deposits** along the glomerular basement membrane.

Raji cell assay are used to quantitate immune complexes.

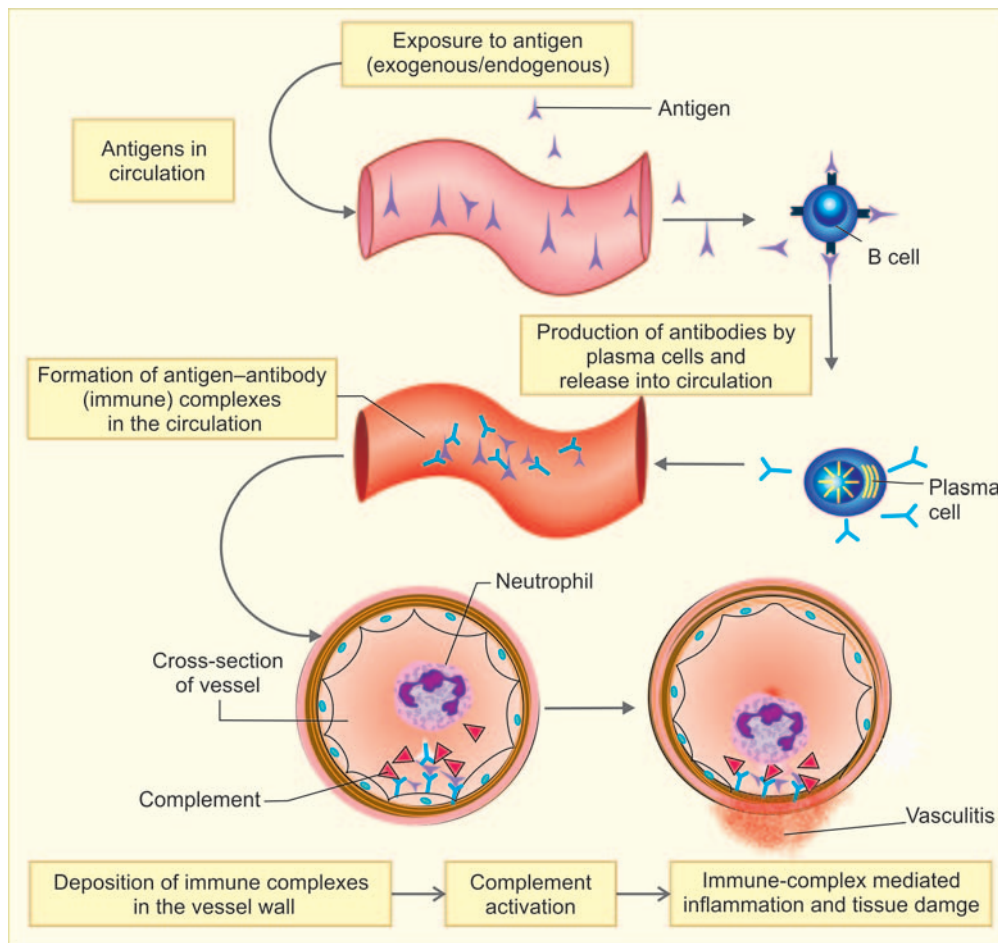


Fig. 6.9: Pathogenesis of systemic immune complex-mediated disease (type III hypersensitivity). Immune complexes are deposited in tissue activate complement system and recruit tissue-damaging inflammatory cells. The pathogenic ability of immune complexes to mediate tissue injury depends on size, solubility, net charge and ability to fix complement

Type III hypersensitivity reactions are immune complex-mediated injuries.

Type III hypersensitivity reactions: Antigen antibody complexes are either formed in the circulation or in situ.

Type III hypersensitivity: Small quantity of immune complexes is formed during normal immune responses and is usually destroyed by phagocytosis.

Very large immune complexes (with antibody excess) are cleared from circulation by macrophages in the spleen and liver. They are usually harmless.

Fibrinoid necrosis: Seen in—

- | | |
|---------------------------|-----------------------|
| 1. Polyarteritis nodosa | 4. Arthus reaction |
| 2. Malignant hypertension | 5. SLE |
| 3. Aschoff bodies | 6. Rheumatoid nodule. |

- **Membranous glomerulonephritis, polyarteritis nodosa** and several other vasculitides.

Type III hypersensitivity reactions: Inflammatory cells, complement, and accompanying release of potent inflammatory mediators is responsible for injury.

Fate of the Lesion

- **Single dose of antigen:** If the disease is due to a single large dose of antigen, the lesions tend to be **self-limiting and lesions resolve**. This is because continued rise in antibody produces larger immune complexes, which are catabolized by phagocytosis. Example: acute serum sickness, perhaps **acute poststreptococcal glomerulonephritis**.
- **Repeated dose of antigen:** A chronic form of serum sickness results from repeated or prolonged exposure to an antigen. Examples:
 - **Systemic lupus erythematosus (SLE)**, which is associated with persistent antibody responses to autoantigens.

Local Immune Complex Disease—Arthus Reaction

- Arthus reaction is a **local area of tissue necrosis usually in the skin**, resulting from acute immune complex vasculitis.
- Arthus reaction can be experimentally produced by intracutaneous injection of an antigen to a previously immunized animal (with circulating antibodies against the antigen). As the antigen diffuses into the vascular wall, it locally binds to the antibody and form large immune complexes at the site of injection.

- Immune complexes deposited in the vessel walls, cause fibrinoid necrosis and thrombosis leading to ischemic injury.

T-CELL MEDIATED (TYPE IV) HYPERSENSITIVITY REACTIONS

Q. Write short note on type IV hypersensitivity reactions/delayed hypersensitivity reactions.

- Type IV hypersensitivity reaction is mediated by T lymphocytes including CD4⁺ and CD8⁺ T-cells.
- It develops in response to antigenic exposure in a previously sensitized individual.
- Reaction is delayed by 48–72 hours after exposure to antigen. Hence also called as delayed-type hypersensitivity (DTH).
- This hypersensitivity reaction is involved in several autoimmune diseases (e.g. rheumatoid arthritis, Hashimoto's thyroiditis), pathological reactions to environmental chemicals (e.g. poison ivy, nickel) and persistent microbes (e.g. tuberculosis, leprosy).

Types: Two types, namely (1) Cytokine-mediated inflammation in which CD4⁺ T-cells produce cytokines and (2) Direct cell toxicity mediated by CD8⁺ T-cells.

Cytokine Mediated Inflammation Elicited By CD4⁺ T-cells (Fig. 6.10)

A. First exposure to antigen

- **Type of antigen:** Antigen may be either exogenous environmental antigens or endogenous (self-antigens causing autoimmune disease).
- **Processing of antigen:** Upon exposure to an antigen, it should be processed by the antigen presenting cells (dendritic cells or macrophages) before presenting it to T-cells, because T-cells cannot directly recognize the antigen.
- **Recognition of antigen by naïve CD4⁺ T-cells in association with class II MHC molecules** on antigen presenting cell (APC).
- **Differentiation of CD4⁺ T-cells:**
 - If the APCs secrete IL-12, the naïve CD4⁺ T-cells differentiate into effector cells of T_H1 type.
 - If the APCs secrete IL-1, IL-6, or IL-23 (instead of IL-12), the naïve CD4⁺ T-cells differentiate into effector cell of T_H17 type.

B. On repeat exposure to an antigen: Previously activated T-cells recognize the antigen presented by APCs. Depending on the cytokines produced, one of the two effector cells, i.e. either T_H1 or T_H17 cells respond.

- T_H1 cells → production of cytokines (e.g. IFN-γ and TNF). IFN-γ (most powerful macrophage activating cytokine) → activates macrophages.
 - Activated macrophages have increased phagocytic and microbicidal power. They secrete IL-12 which amplify the T_H1 response.
- T_H17 cells: They are activated by some microbial antigens as well as self-antigens in autoimmune diseases. They produce IL-17, IL-22, chemokines and other cytokines. These cytokines promote inflammation by recruiting more neutrophils and monocytes to the site of reaction.

IL-2 is characteristic product in T_H1 response.

Tuberculin Reaction (Montoux Test)

- Tuberculin reaction is a classical example for delayed-type hypersensitivity.
- It is produced by the intracutaneous injection of purified protein derivative (PPD, also called tuberculin), a protein-containing antigen of the tubercle bacillus.
- In a previously sensitized individual, the injection site becomes red and indurated in 8–12 hours, reaches a peak (usually 1–2 cm in diameter) in 24–72 hours, and thereafter slowly subsides.
- Microscopically, the injected site shows perivascular accumulation “cuffing” of CD4⁺ T-cells and macrophages.

Granuloma

Prolonged DTH reaction against persistent microbes (e.g. tubercle bacilli) or other nondegradable (foreign bodies) injurious agent may produce a special microscopic reaction known as **granulomatous inflammation**.

Mechanisms of granuloma formation in cell-mediated (type IV) hypersensitivity reactions (Fig. 6.11): Different step involved are:

- **Exposure to antigen.**
- **Processing of antigen by the antigen presenting cells (APCs)** (dendritic cells or macrophages).
- **Presenting antigen to and its recognition by naïve CD4⁺ T-cells**, in association with class II MHC molecules on APC.
- **Differentiation, proliferation and perivascular accumulation of CD4⁺ T-cells.**
- **Replacement of CD4⁺ T-cells by activated macrophages over a period of 2 or 3 weeks.**
- **TNF secreted by activated macrophages causes recruitment of monocytes from circulation.**
- The activated macrophages undergo a morphologic evidence of activation. These include—transformation into large, flat, eosinophilic and epithelium-like cells

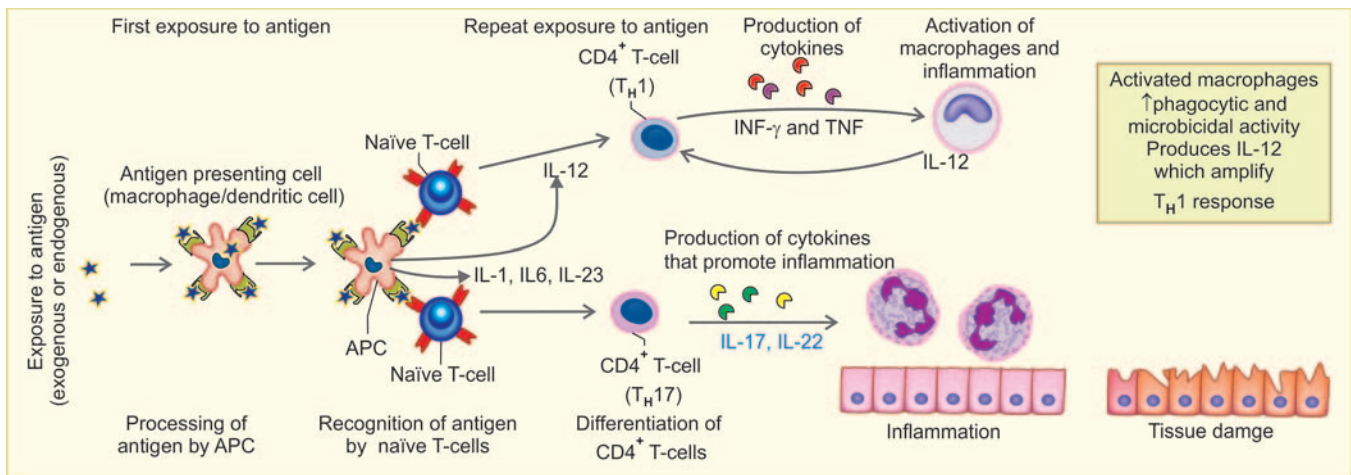


Fig. 6.10: Mechanisms of CD4⁺ T-cell mediated (type IV) hypersensitivity reactions. In delayed-type hypersensitivity reactions, antigens are phagocytized, processed by APC (antigen presenting cells, e.g. dendritic cell, macrophage). They are presented to naïve T-cells. Depending on the cytokines produced by APC, naïve T-cells may differentiate into CD4⁺ T_H1 or CD4⁺ T_H17. CD4⁺ T_H1 cells secrete cytokines that activate macrophage leading to tissue injury. CD4⁺ T_H17 cells produce cytokines that produce inflammation by recruiting neutrophils. Both mechanisms produce tissue damage

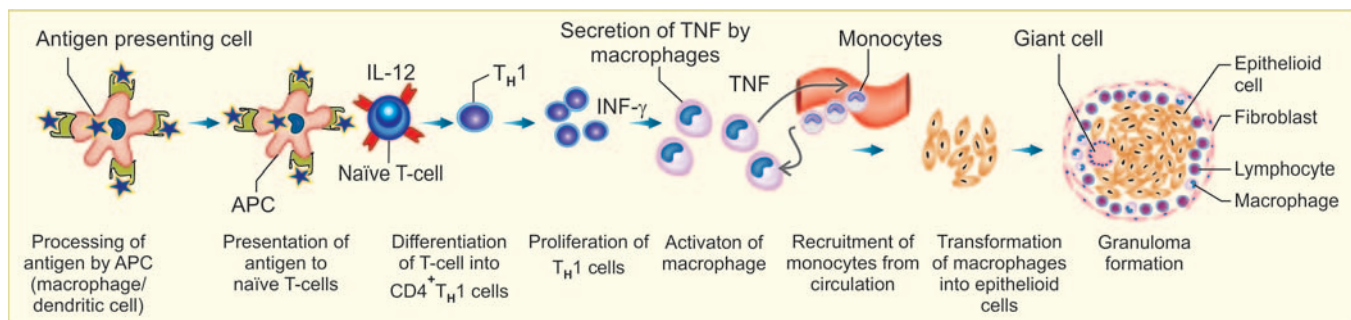


Fig. 6.11: Mechanisms of granuloma formation in cell-mediated (type IV) hypersensitivity reactions

referred to as **epithelioid cells**. The cytokines (e.g. INF-γ) may cause fusion of epithelioid cells to form multinucleated **giant cells**.

- **Granuloma** is a microscopic aggregate of epithelioid cells (Fig. 6.12), surrounded by a rim of lymphocytes. Older granulomas are enclosed by rim of fibroblasts and connective tissue.

Positive tuberculin test indicates that the individual is previously exposed to tuberculosis. However, immunosuppression (e.g. HIV) may be associated with negative tuberculin test despite the presence of severe infection.

Contact Dermatitis

Contact with various environmental antigens (e.g. poison ivy, metals such as nickel and chromium, chemicals like hair dyes, cosmetics, soaps) may evoke inflammation with

blisters in the skin at the site of contact known as contact dermatitis.

Direct Cell Toxicity Mediated By CD8⁺ T-cells

It is a type of T-cell mediated tissue injury due to CD8⁺ T lymphocytes (also called as cytotoxic T lymphocytes or CTLs), which kill antigen-bearing target cells. For example, killing of virus infected cells (e.g. in viral hepatitis) and some tumor cells.

Mechanism of Cytotoxic T-cell Mediated Killing

In this type of hypersensitivity, CD8⁺ cytotoxic T-cells kill antigen-bearing target cells by two mechanisms:

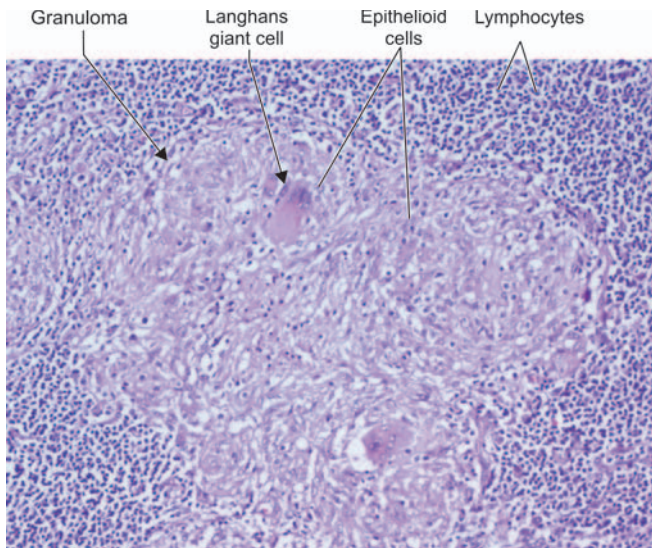


Fig. 6.12: Granulomatous inflammation. Section of a lymph node with granuloma. It consists of an aggregate of epithelioid cells surrounded by lymphocytes. The granuloma shows several multinucleate giant cells

CD4⁺ T-cells:

1. Detects MHC II
2. But it expresses MHC I because it is a nucleated cell.

CD8⁺ T-cells:

1. Detects MHC I
2. Expresses MHC I.

INF- γ activated macrophages:

- Increased phagocytic and microbicidal power
- Express more class II MHC molecules on the surface→ facilitate further antigen presentation
- Secrete TNF, IL-1 and chemokines→ promote inflammation
- Produce more IL-12→ amplify T_H1 response.

1. **Perforin-granzymes system** (Fig. 6.13A): Main mechanism of T-cell mediated killing of target cells.
 - CTLs have lysosome-like granules containing preformed mediators **perforins** and **granzymes**.
 - CTLs that recognize the target cells secrete perforin and granzymes.
 - **Perforin** is a transmembrane **pore-forming molecule**, which allows the entry of granzymes into the cytoplasm of target cells.
 - **Granzymes** are proteases, which **cleave and activate cellular caspases** (effector pathway of apoptosis).
 - Activated caspases induce **apoptosis of the target cells**.
2. **Through Fas ligand** (Fig. 6.13B): Activated CTLs also express Fas ligand (a molecule with homology to TNF),

which can bind to Fas expressed on target cells and cause **apoptosis by extrinsic pathway**.

Examples of T-cell mediated (type IV) hypersensitivity are shown in Table 6.7.

Salient features and differences between hypersensitivity reactions are presented in Table 6.8.

Type IV hypersensitivity: CD8⁺ cytotoxic T-cells (CTLs) kill cells (by apoptosis) that express antigens in the cytoplasm that are seen as foreign. Example: virus infected cells, tumor cells and donor graft cells.

AUTOIMMUNE DISEASES

Definition: Autoimmunity is defined as **immune reactions** in which **body produces autoantibodies** and **immunologically competent T lymphocytes against self-antigens**.

Autoimmunity is an important cause of certain diseases in humans (Table 6.9).

- **Organ-specific disease:** It may be restricted to a single organ or tissue (e.g. type 1 diabetes).
- **Systemic or generalized disease:** For example, systemic lupus erythematosus (SLE).
- **Involving more than one organ:** For example, Goodpasture syndrome, in which lung and kidney are involved.

Normal individuals are unresponsive (tolerant) to their own (self) antigens and autoimmune disorders results from the loss of self-tolerance.

Autoimmune diseases: May be mediated by:

1. Autoantibodies or
2. T-cells against self-antigens.

Autoimmunity: Presence of immune responses against self tissue. Autoimmune diseases occur if these immune responses cause significant tissue/organ damage.

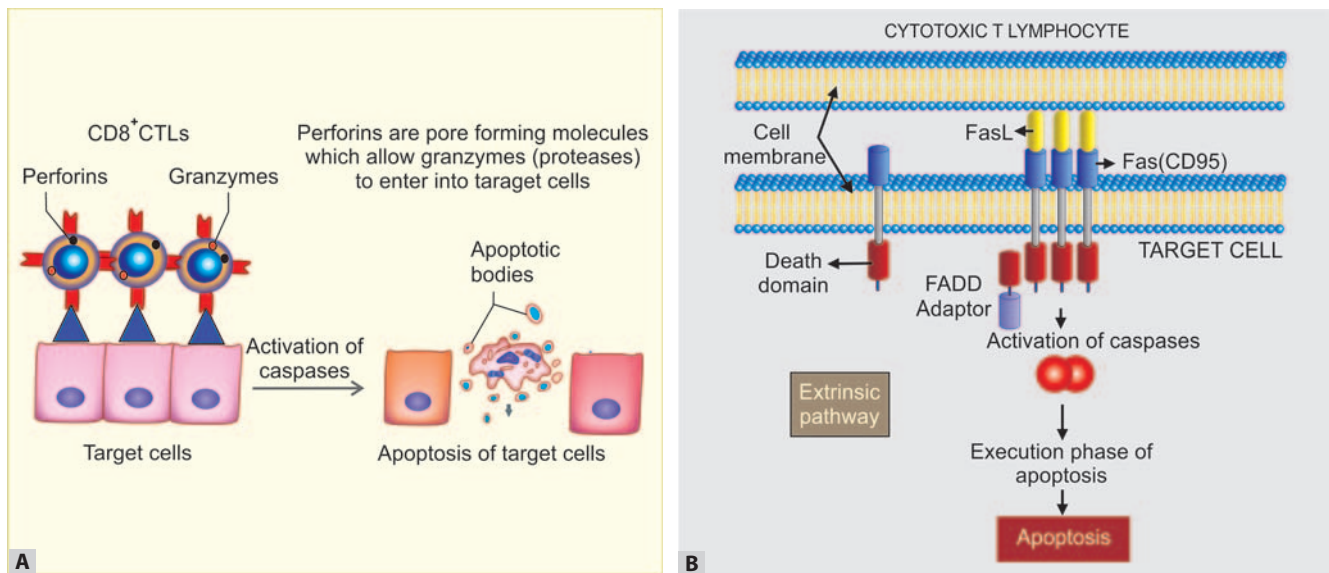
IMMUNOLOGICAL TOLERANCE

Immunological tolerance is the phenomenon in which there is **no immune response to specific (usually self) antigens**. It is the result of exposure of lymphocytes to that specific antigen.

Immunological tolerance: Unresponsiveness to self-antigen is of two types:

1. Central tolerance
2. Peripheral tolerance.

Self-tolerance: It is absence of immune response to an individual's own antigens.



Figs 6.13A and B: (A) Mechanisms of T-cell mediated (type IV) hypersensitivity reactions by direct cell toxicity mediated by CD8⁺ cytotoxic T lymphocytes; (B) Cytotoxic cell-mediated killing of target cells through Fas ligand

TABLE 6.7: Examples of T-cell mediated (type IV) hypersensitivity

Disease	Antigen	Manifestations
Type 1 diabetes mellitus	Antigens of pancreatic islet β cells (insulin, glutamic acid decarboxylase, others)	Insulitis (chronic inflammation in islets), destruction of β cells; diabetes mellitus
Rheumatoid arthritis	Collagen; citrullinated self-protein	Chronic arthritis, inflammatory destruction of articular cartilage and bone
Inflammatory bowel disease	Enteric bacteria, self-antigen	Chronic inflammation of intestine, ulceration
Hashimoto thyroiditis	Thyroglobulin and other thyroid proteins	Hypothyroidism
Contact sensitivity (dermatitis)	Environmental chemicals (e.g. poison ivy)	Inflammation of skin and blisters

Most potent stimulator of naïve T-cell is mature dendritic cell.

Mechanisms of Self-tolerance

- Numerous different antigen receptors are produced in the developing T and B lymphocytes.
- These receptors are capable of recognizing self-antigens and these lymphocytes have to be eliminated or inactivated as soon as they recognize the antigens, to prevent immune reaction against own antigens.
- The mechanism by which this is achieved can be broadly classified into two groups: (1) central tolerance and (2) peripheral tolerance (Fig. 6.14).

Central Tolerance (Fig. 6.14)

Q. Write short note on central immune tolerance.

It is the process by which self-reactive T and B lymphocytes (which recognize self-antigens) are deleted (killed) during their maturation within the central (or generative) lymphoid

organs. These organs are thymus for T-cells and the bone marrow for B-cells.

Central tolerance: Self-reactive lymphocytes that recognize self antigens are killed by apoptosis in the central lymphoid organs.

Mechanisms of Central Tolerance

• T-cells:

- Negative selection or deletion:** It is a process by which immature self-reactive T lymphocytes that encountered antigens are eliminated by apoptosis. It occurs in the thymus. AIRE (autoimmune regulator) is a protein product of AIRE gene is critical for deletion of immature self-reactive T-cells. Mutations in AIRE gene are the cause of an autoimmune polyendocrinopathy.
- Regulatory T-cells:** Some T-cells may differentiate into regulatory T-cells.

TABLE 6.8: Salient features and differences between hypersensitivity reactions

Features	Type I	Type II	Type III	Type IV
Antigens	Exogenous allergens include: Pollen, moulds, mites, drugs, food, etc.	Cell surface or tissue bound	Soluble exogenous (viruses, bacteria, fungi, parasites) or endogenous autoantigens	Cell/tissue bound
Antibody involved	IgE	IgG and IgM	IgG, IgM, IgA	None
Mediators	From mast cells	Complement and lymphokines	Complement	T lymphocytes, activated macrophages
Time taken for reaction to develop	5–10 min	6–36 hours	4–12 hours	48–72 hours
Immunopathology	Edema, vasodilatation, mast cell degranulation, eosinophils	Antibody-mediated damage to target cells/tissue	Acute inflammatory reaction, neutrophils, vasculitis	Perivascular inflammation, mononuclear cells, fibrin, granulomas caseation and necrosis in TB
Examples of diseases and conditions produced	<ul style="list-style-type: none"> • Asthma (extrinsic) • Urticaria/edema • Allergic rhinitis • Food allergies • Anaphylaxis 	<ul style="list-style-type: none"> • Autoimmune hemolytic anemia • Transfusion reactions • Hemolytic disease of newborn • Goodpasture syndrome • Acute rheumatic fever • Pernicious anemia • Myasthenia gravis 	<ul style="list-style-type: none"> • Autoimmune, e.g. SLE • Glomerulonephritis • Rheumatoid arthritis • Farmer's lung disease • Hypersensitivity pneumonitis • Arthus reaction (localized) 	<ul style="list-style-type: none"> • Pulmonary TB • Contact dermatitis • Tuberculin test • Leprosy • Graft-versus-host

Q. List autoimmune diseases.**TABLE 6.9:** Examples of autoimmune diseases

Diseases mediated by antibodies	Diseases mediated by T-cells
Organ-specific	Organ-specific
Autoimmune hemolytic anemia	Type 1 diabetes mellitus
Autoimmune thrombocytopenia	Hashimoto thyroiditis
Goodpasture syndrome	Crohn's disease
Myasthenia gravis	Multiple sclerosis
Graves' disease	
Systemic	Systemic
Systemic lupus erythematosus (SLE)	Rheumatoid arthritis

- **B-cells:**
 - **Apoptosis:** Immature **B-cells** that recognize self-antigens may also undergo **apoptosis in the bone marrow**.
 - **Receptor editing:** It is a process by which some self-reactive **B-cells** undergo rearrangement of antigen receptor genes and express new receptors. These receptors are no longer self-reactive.

Peripheral Tolerance (Fig. 6.14)

Silencing of potentially autoreactive T- and B-cells in peripheral tissues is called as peripheral tolerance.

Mechanisms of Peripheral Tolerance

1. **Anergy:** It refers to functional **inactivation of autoreactive lymphocytes** in the peripheral tissues.

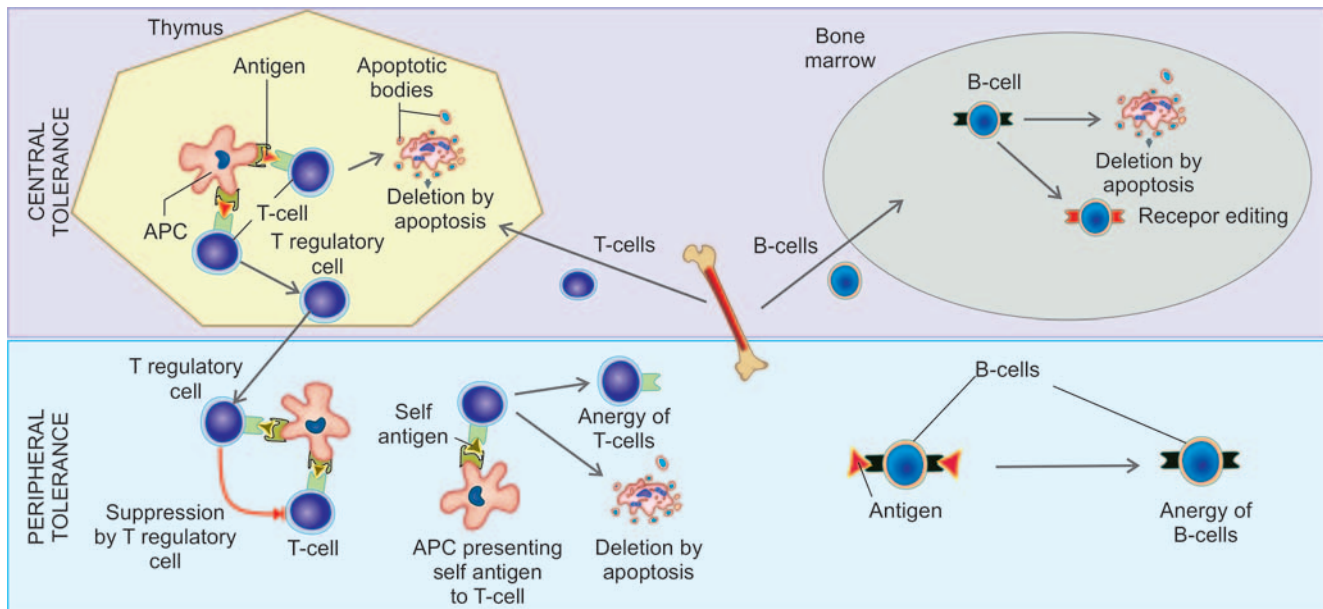


Fig. 6.14: Main mechanisms of central and peripheral immunological self-tolerance
Abbreviation: APC, antigen-presenting cell.

- **Anergy of T-cells:** Normally, activation of T-cells require two signals from antigen presenting cells (APCs): (1) **peptide antigen** on the surface of APCs and (2) **co-stimulatory signals** ("second signals").
 - If the antigen is presented by APCs without co-stimulatory signals, a negative signal is delivered by APCs to the antigen-specific T-cells and the T-cell becomes inactive (i.e. anergic).
 - **Anergy of B-cells:** It may develop, if B-cells encounter self-antigen in the absence of specific helper T-cells.
2. **Suppression by regulatory T-cells:** It plays a major role in preventing immune reactions against self-antigens.
 3. **Activation-induced cell death:** It is a mechanism in which **apoptosis of mature activated self-reactive lymphocytes** is produced. Apoptosis may be by **intrinsic (mitochondrial) pathway** or by **extrinsic pathway** (refer Chapter 1).

Peripheral tolerance: Autoreactive lymphocytes that recognize self-antigens in peripheral tissues are inactivated (anergy) or suppressed by regulatory T-cells or undergo apoptosis.

A super-antigen is a bacterial product that binds to beta chain of TCR and MHC class II molecules of APC simulating T-cell activation.

Type I MHC presents peptide antigen to T-cell, so that peptide binding site is formed by distal domain α 1 and 2.

MECHANISMS OF AUTOIMMUNITY (FIG. 6.15)

Q. Mechanism of autoimmune disorders.

- Breakdown of self-tolerance may lead to autoimmunity.
- The mechanism of autoimmunity may be the result of combination of the two main factors, namely (1) **genetic** and (2) **environmental factors**.

Autoimmunity: Due to breakdown of tolerance.

Genetic Factors

Role of susceptibility genes: Most autoimmune diseases are complex multigenic disorders and genetic factors have an important role.

- **Runs in families:** The incidence is greater in monozygotic than in dizygotic twins.
- **Association with HLA genes:** It is most significant.

Environmental Factors

A. **Role of Infections:** A variety of microbes may trigger autoimmunity by several mechanisms.

- **Molecular mimicry:** Few viruses and microbes may express antigens that have the same amino acid

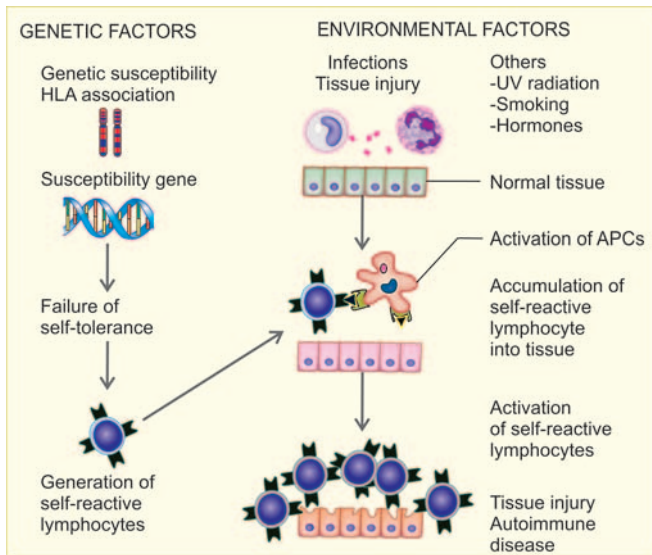


Fig. 6.15: Pathogenesis of autoimmunity

sequences as self-antigens. Immune responses against them may attack self-tissue and this phenomenon is known as molecular mimicry. For example, **rheumatic heart disease** in which antibodies formed against streptococcal bacterial proteins cross-react with myocardial proteins and cause myocarditis.

- **Breakdown of anergy: Tissue necrosis and inflammation produced by microbial infections can cause up-regulation of costimulatory molecules on APCs. This may favor breakdown of anergy and activation of T-cells.**

B. Other environmental factors:

- Ultraviolet radiation
- Cigarette smoking
- Local tissue injury
- Hormones.

HLA class III region genes: Important elements in governing susceptibility to autoimmunity.

HLA typing is useful in:

- Organ transplant
- Disputed paternity.

SYSTEMIC LUPUS ERYTHEMATOSUS

Systemic lupus erythematosus (SLE) is a **chronic autoimmune disease** having following characteristics:

1. **Protean manifestation and variable behavior.**
2. **Remission and relapses.**

Mixed lymphocyte culture is used to identify: MHC class II antigen.

HLA class III region genes are important elements in governing susceptibility to autoimmune disease.

Feature common to both cytotoxic T-cells and NK cells is that they are effective against virus infected cells.

Anti-topoisomerase I is marker of: Systemic sclerosis.

3. **Multisystemic involvement:** Mainly affects **skin, kidneys, joints, serous membranes and heart.**
4. **Broad spectrum of autoantibodies**, most important is antinuclear antibodies (ANAs).

SLE: Systemic autoimmune disease caused by autoantibodies against numerous self-antigens and forms immune complexes.

SLE: Term lupus is derived from Latin for wolf, because of the skin lesion on the face looked as though eaten by a wolf.

Q. Write short note on etiology and pathogenesis of SLE.

Etiology

Systemic lupus erythematosus is an **autoimmune disease** in which fundamental defect is **failure of self-tolerance**. It leads to production of **many autoantibodies** that **damage the tissue either directly or indirectly by depositing immune complex deposits**. A combination of genetic and environmental factors plays a role in the pathogenesis of SLE.

Genetic Factors

Evidence to support genetic predisposition are:

1. **Familial association:**
 - **Family members** of SLE patients have an **increased risk** of SLE. About 20% of unaffected first-degree relatives may show autoantibodies.
 - **High rate** of concordance (>25%) in **monozygotic twins** when compared with dizygotic twins (1–3%).

2. **HLA association:** Risk is more with HLA-DR2 or HLA-DR3.
3. Other genetic factors:
 - Genetic deficiencies of early complement components (such as C2, C4 or C1q): It may result in—(1) impaired removal of circulating immune complexes by the mononuclear phagocyte system, (2) defective phagocytic clearance of apoptotic cells and (3) failure of B-cell tolerance. If apoptotic cells are not cleared, their nuclear components may elicit immune responses.
 - Polymorphism in the inhibitory F_c receptor → inadequate control of B-cell activation.
3. **Failure of B-cell tolerance:** Occurs due to **defects in both central** (i.e. bone marrow) and **peripheral tolerance** → **higher autoreactive B-cells**.
4. $CD4^+$ helper T-cells specific for nucleosomal antigens: These escape tolerance and produce high-affinity pathogenic autoantibodies.

SLE: Complex disorder of multifactorial origin which results from interactions of genetic, immunological and environmental factors.

SLE:

1. Genetic factors
2. Environmental factors
3. Immunological abnormalities.

Environmental Factors

1. **Ultraviolet (UV) radiation:** Exposure to sunlight exacerbates the lesions of the disease.
 - **Mechanism:** UV irradiation → causes **apoptosis** of host cells → **increases burden of nuclear antigens** and **promote inflammation**.
2. **Cigarette smoking:** It is associated with development of SLE.
3. **Sex hormones:** SLE is 10 times greater during the reproductive period (17 through 55 years) **in women** than in men. SLE shows exacerbation during normal menses and pregnancy.
4. **Drugs:** Examples include **hydralazine, procainamide, isoniazid and D-penicillamine** can produce SLE-like disease and disease remits after withdrawal of the drug.

Immunological Abnormalities

Several immunological abnormalities of both innate and adaptive immune system have been observed in SLE.

1. **Type I interferons:**
 - These are antiviral cytokines normally produced by B-cells during innate immune responses to nucleic acid of viruses.
 - $INF-\alpha$ is a type I interferon produced by plasmacytoid dendritic cells and **large amounts is produced** in SLE. It may indirectly produce autoantibodies.
2. **Toll-like receptor (TLR) signals:**
 - TLRs present in B lymphocytes normally sense microbial products, including nucleic acids.
 - In SLE, nuclear DNA and RNA within the **immune complexes may activate B lymphocytes by engaging with TLRs**. These **activated B-cells** specific for nuclear antigens may **produce antinuclear autoantibodies**.
3. **Increased apoptosis triggered by environmental agents:** UV irradiation and other environmental agents may cause death of cells by **apoptosis**.
4. **Inadequate clearance of apoptotic bodies:** It results in accumulation of large amount of nuclear antigens. It is partly due to defect in complement proteins.
5. **Susceptibility genes with failure of self-tolerance:** Genetic abnormality in B and T lymphocytes is responsible for failure of self-tolerance.
6. **Stimulation of self-reactive B-cells:** It produces antibodies against the self-nuclear antigens.
7. **Formation of antigen–antibody (immune) complexes** in the circulation.
8. **Endocytosis of immune complexes:** The antibody portion of immune complexes bind to F_c receptors on B-cells and dendritic cells (DCs) and the immune complexes may be internalized by endocytosis.
9. **TLR engagement by nuclear antigens:** Nucleic acid components of immune complexes bind to TLRs of B-cells and DCs.
10. **TLR stimulation of B-cells and DCs:** Binding to TLR—
 - Stimulate B-cells to produce autoantibodies.
 - Activate dendritic cells (mainly plasmacytoid DCs) to produce $INF-\alpha$ → stimulate B- and T-cells to further amplify immune response → cause more apoptosis.
11. **Persistent production of autoantibodies:** Thus, a cycle of antigen release and immune activation → results in the persistent production of IgG autoantibodies.

Pathogenesis of SLE (Fig. 6.16)

Different steps are:

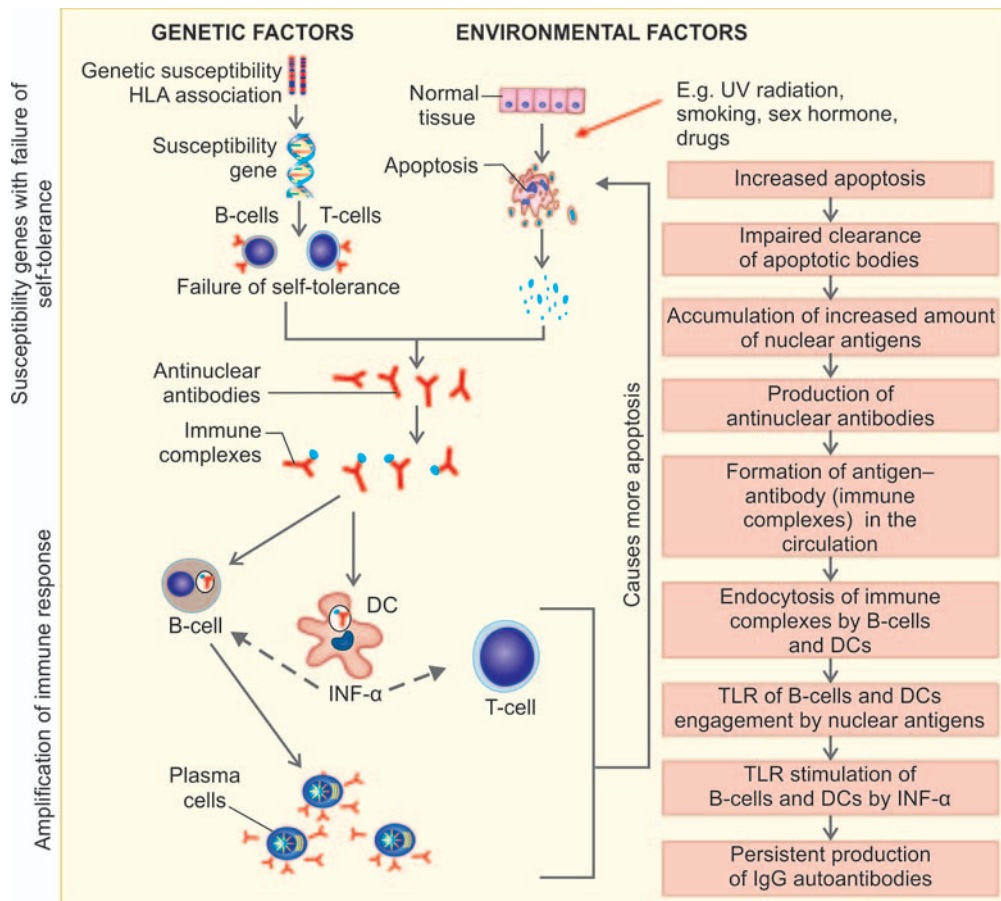


Fig. 6.16: Pathogenesis of systemic lupus erythematosus
Abbreviation: TLRs, Toll-like receptors; DCs, Dendritic cells

Autoantibodies in SLE

Q. Write short note on antibodies in SLE.

SLE is characterized by the production of **several diverse autoantibodies**. Some antibodies are against different nuclear and cytoplasmic components of the cell that are not organ specific. Other antibodies are directed against specific cell surface antigens of blood cells.

Importance of autoantibodies: (1) **diagnosis and management** of patients with SLE and (2) responsible for **pathogenesis of tissue damage**.

Types of Antibodies

SLE: Caused by autoantibodies against numerous self-antigens, major being antinuclear antibodies (ANAs).

Antinuclear Antibodies (ANAs)

They are directed against various nuclear antigens including DNA, RNA and proteins (all together called generic ANAs) and can be grouped into different categories (Table 6.10).

Other Autoantibodies

- **Autoantibodies against blood cells**, namely (1) red cells, (2) platelets, (3) neutrophils and (4) lymphocytes.

Q. Write short note on antiphospholipid antibody

- **Antiphospholipid antibodies aPL** are detected in 40–50% of SLE patients but they are not specific for SLE.
 - The term **antiphospholipid antibody** is misleading, because these antibodies react with plasma proteins of complexes rather than directly with phospholipids (Fig. 6.17).
 - Antiphospholipid antibody includes **lupus anticoagulant antibody**, **anticardiolipin antibody** and **anti-β₂ glycoprotein antibody**.
 - **Complications:** These autoantibodies can lead to → **increased venous and arterial thrombosis and thrombocytopenia** → **recurrent spontaneous miscarriages** and **focal cerebral or ocular ischemia**.
 - **Antibodies against phospholipid-β₂-glycoprotein complex** also bind to cardiolipin antigen. Since cardiolipin antigen is used in the serological test

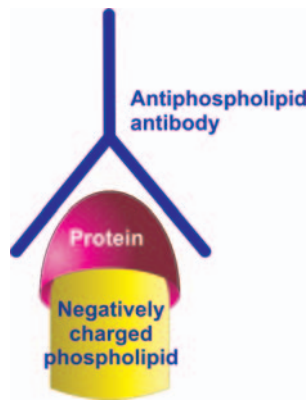


Fig. 6.17: Antiphospholipid antibody against plasma proteins bound to phospholipids

TABLE 6.10: Important antinuclear antibodies and their clinical utility

Type of antinuclear antibodies	Antigen recognized	Clinical utility
Anti-dsDNA*	DNA (double-stranded)	High titers of IgG antibodies are SLE-specific (but not to single-stranded DNA)
Anti-Smith*	Nonhistone proteins bound to RNA	Specific for SLE; do not usually correlate with disease activity or clinical manifestations
Antihistone antibodies	Histones associated with DNA	More frequent in drug-induced lupus than in SLE
Antibodies to DNA	Multiple nuclear	Best screening test; if repeated test are negative SLE unlikely
Anti-Ro (SS-A)	RNP (ribonucleoprotein)	Not specific for SLE; predictive value indicates increased risk for neonatal lupus and sicca syndrome

*Antibodies specific to SLE

Females with child-bearing potential and SLE should be screened for aPL and anti-Ro.

Antibodies specific to SLE (Confirmatory tests): Antibody to—
 1. Double-stranded DNA (dsDNA)
 2. Spliceosomal proteins Smith (Sm) antigen.

Serum ANA: Screening test for SLE.

Antiphospholipid syndrome: Increased risk for venous or arterial clotting and fetal loss.

for syphilis, SLE patients may give a false-positive serological reaction for syphilis.

- **Two tests that measure different antibodies (anticardiolipin and the lupus anticoagulant):** (1) ELISA for anticardiolipin and (2) a sensitive phospholipid-based activated prothrombin time, such as the dilute Russell viper venom test.

Anticardiolipin antibodies in SLE may produce false +ve VDRL test for syphilis.

Mechanisms of Tissue Injury

Autoantibodies mediate tissue injury.

- **Type III hypersensitivity:** It occurs with deposition of immune complexes. It is the most common cause of tissue injury and visceral lesions.
- **Type II hypersensitivity:** Autoantibodies against cell surface antigens specific for RBCs, white cells and platelets → opsonize these cells → promote their phagocytosis and lysis → **cytopenias**.

SLE: Shows features of both type II (hematological abnormalities) and type III (visceral lesions) hypersensitivity reactions.

LE Bodies or Hematoxylin Bodies

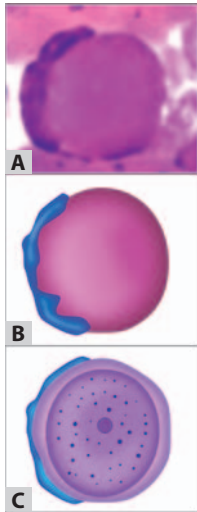
Q. Write short note on LE cell and its associated conditions.

LE Bodies

- ANAs cannot penetrate intact cells, but if nuclei of the cell are exposed, they can bind to them.
- In tissues, nuclei of damaged cells react with ANAs, lose their chromatin pattern, and appear homogeneous, to produce **LE bodies or hematoxylin bodies**.

LE Cell (Fig 6.18 and refer page 344)

- It is related to LE bodies and can be demonstrated in vitro.
- The blood sample is agitated to damage the nucleated cells and it releases the nuclei.
- The nuclei of damaged cells react with ANAs to form a homogenous denatured nuclear material.
- The LE cell is any phagocytic leukocyte (blood neutrophil or macrophage) that has engulfed this denatured nucleus of an injured cell.
- The demonstration of LE cells in vitro was used as a test for SLE.
- With the advent of new techniques for detection of ANAs, this test is of only historical interest.
- Sometimes, LE cells can be found in body fluid such as pericardial or pleural effusions.



SLE: Subendothelial immune complex deposits give rise to "wire-loop" lesions on light microscopy.

Figs 6.18A to C: Appearance of LE cell, (A) Leishman stain, (B) diagrammatic and (C) tart cell

- Interpretation: LE cell is positive in about 70% of SLE. It may also be positive in conditions such as rheumatoid arthritis, lupoid hepatitis, penicillin sensitivity, etc.

LE cell: Phagocytic leukocyte (neutrophil or macrophage) that has engulfed the denatured nucleus of an injured cell.

Tart cell: Usually monocyte that has ingested another cell or nucleus of another cell.

MORPHOLOGY

SLE is a systemic autoimmune disease and morphologic changes in SLE are **extremely variable**.

The most **characteristic lesions** of SLE are **due to deposition of immune complexes** in **blood vessels, kidneys, connective tissue, and skin**.

Kidney

Kidney may be **involved in about 50%** of SLE patients and is one of the most important organs involved.

Pathogenesis of glomerulonephritis: Immune complexes composed of **DNA and anti-DNA antibodies** get deposited in the glomeruli → inflammation → proliferation of cells (endothelial, mesangial and/or epithelial).

Morphologic classification of lupus nephritis: Six patterns are recognized but none of these are specific for SLE.

1. **Minimal mesangial** lupus nephritis (class I): It is characterized by **immune complex deposition in the mesangium** granular deposits of immunoglobulin and complement and no recognizable structural changes by light microscopy.
2. **Mesangial proliferative** lupus nephritis (class II): It is characterized by **immune complex deposition in the mesangium** and **mild-to-moderate increase in mesangial cells and mesangial matrix**.

3. **Focal proliferative** lupus nephritis (class III): It is seen in 20–35% of patients. The lesions are **focal and may be segmental** (affecting only a portion of the glomerulus) or **global** (involving the entire glomerulus). Affected glomeruli may show **proliferation of endothelial and mesangial cells, or parietal epithelial cells** (crescent formation), **fibrinoid necrosis, leukocyte infiltration, and eosinophilic deposits or intracapillary thrombi**.
4. **Diffuse proliferative** lupus nephritis (class IV): It is severe form and occurs in 35–60% of patients. Lesions are **diffused** (>50% of glomeruli) and most of involved glomeruli may show **proliferation of endothelial, mesangial and epithelial cells**. The proliferation of parietal epithelial cells may produce cellular crescents. **Prominent, subendothelial deposits cause homogeneous thickening of the capillary wall**, which on light microscopy appear as a "wire-loop" lesion (Fig. 6.19). These wire loops **may be seen in both focal and diffuse proliferative (class III or IV) lupus nephritis**.
5. **Membranous** lupus nephritis (class V): It is seen in 10–15% of patients and is characterized by **diffuse thickening of the capillary walls** similar to idiopathic membranous glomerulonephritis → nephrotic syndrome.
6. **Advanced sclerosing** lupus nephritis (class VI): It shows sclerosis of more than 90% glomeruli.

Interstitial and tubules: They may show changes, but are usually not dominant abnormality.

Immunofluorescence: It shows granular deposits of antibody and complement.

Electron microscopy: It shows electron-dense deposits (immune complexes) in mesangial, intramembranous, subepithelial, or subendothelial locations.

Wire loop lesions: Seen in diffuse proliferative glomerulonephritis (class IV) in SLE. It may also be seen in focal lupus nephritis (class III).

Vegetations:

1. Larger in infective endocarditis.
2. Smaller (verrucae), seen at the lines of closure of the valve leaflet in rheumatic heart disease.
3. Single or multiple warty deposits on either surface of the leaflets of any heart valves in SLE.

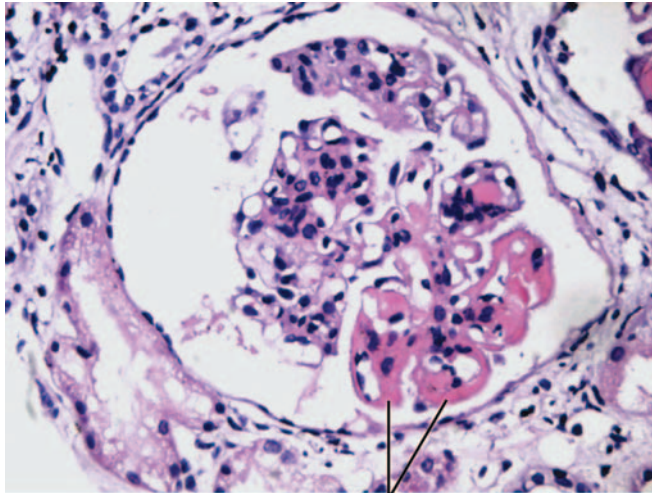
Blood Vessels

An **acute necrotizing vasculitis** (involving small arteries and arterioles) may be seen in any involved tissue. The arteritis is characterized by **fibrinoid necrosis** in the vessel walls. In **chronic stages**, vessels undergo fibrous thickening of wall and **narrowing of the lumen**.

Heart

Any layer of heart may be involved. **Valvular endocarditis** (Libman-Sacks/**nonbacterial verrucous endocarditis**) appear as **single or multiple 1–3 mm warty deposits on either surface of the leaflets of any heart valves**.

Libman-Sacks endocarditis is seen in SLE.



Subendothelial deposits producing homogeneous thickening of capillary wall (wire-loop)

Fig. 6.19: Lupus nephritis showing glomerulus with "wire-loop" lesions due to extensive subendothelial deposition of immune complexes

SLE: Antigen and antibodies form immune complexes which are responsible for the tissue damage.

Clinical Features

- SLE is a **multisystem** disease with **variable clinical presentation**.
- **Age:** It usually occurs in young woman between **20 and 30 years**, but may manifest at any age.
- **Sex:** It predominantly affects **women**, with female-to-male ratio of 9:1.
- **Onset:** Acute or insidious with fever.
- **Typical presentation:** **Butterfly rash over the face, fever, pain without deformity in one or more peripheral joints, pleuritic chest pain and photosensitivity**. SLE patients are **susceptible to infections**, because of immune dysfunction and treatment with immunosuppressive drugs.

Laboratory Findings

Q. Write short note on laboratory diagnosis of SLE.

- **Purpose:** (1) To establish or rule out the diagnosis, (2) follow the course of disease, and (3) to identify adverse effects of therapies.
- **Tests for autoantibodies (refer page 136 and Table 6.10):** ANAs are found in almost all patients, but it is not specific. Various methods of detecting antibodies include:
 - Indirect immunofluorescence assay (IFA): They can identify ANAs. Significance of IFA assay are:
 - ♦ Extremely sensitive (positive in more than 95%)

- ♦ Limited specificity because it is positive in patients with other autoimmune diseases, chronic infections and cancer.
- Multiplex flow cytometry immunoassay.
- ELISA (for smith antigen).
- **Standard tests for diagnosis:** Includes complete blood count, platelet count, ESR (raised) and urinalysis.
- **Tests for following disease course:** These tests to indicate the status of organ involvement known to be present during SLE flares.
 - **Renal involvement:** Urinalysis may show **hematuria, red cell casts, proteinuria**, or nephrotic syndrome.
 - **Hematologic changes:** Hemoglobin levels (anemia) or platelet counts (thrombocytopenia) and ESR.
 - Serum levels of creatinine or albumin.
 - Decreased complement component levels in serum such as C3 and C4 are often indicators of enhanced consumption and increased disease activity.

Course: It is variable and unpredictable. It shows remissions and exacerbations.

Cause of death: Renal failure and intercurrent infections.

MAJOR HISTOCOMPATIBILITY COMPLEX MOLECULES

- All human cells have a series of molecules on their surfaces that are recognized by other individuals as foreign antigens. **Major histocompatibility complex (MHC) molecules** were discovered as **products of genes that evoke rejection of transplanted organs** and responsible for tissue compatibility between individuals.
- The **human MHC** are commonly called the **human leukocyte antigen (HLA) complex** is the name of the **loci of genes** densely packed (clustered) on a **small segment on chromosome 6 (6p21.3)**. They were named HLA because in humans MHC-encoded proteins were initially detected on leukocytes by the binding of antibodies.
- **Physiologic function of MHC molecules:** To **display peptide fragments of proteins for recognition by antigen-specific T-cells**.
- The MHC molecules are products of MHC gene. The best known of these genes are the HLA class I and class II genes. Their products are important for immunologic specificity and transplantation histocompatibility, and they play a major role in susceptibility to a number of autoimmune diseases.
- **Polymorphism of MHC gene:**
 - **MHC gene is highly polymorphic.** Polymorphism means that there are many alleles of each MHC gene resulting in extreme (high degree) variation in

the MHC in human population (genetic diversity). Each person inherits one set of these alleles that is different from the alleles in most other persons. The possibility of two different individuals having the same combination of MHC molecules is very remote. Therefore grafts exchanged between individuals are recognized as foreign and attacked by the immune system. **Polymorphism is an important barrier in organ transplantation.**

- **HLA haplotype:** It is the combination of HLA alleles in each individual. Each individual inherits one set of HLA genes from each parent and thus typically expresses two different molecules for every locus.
- **Importance of MHC:** (1) In organ/tissue transplantation and (2) HLA is linked to many autoimmune diseases.

MHC is a cluster of genes located on short arm of chromosome 6 (6p21.3).

Tests for detection of HLA:

1. Lymphocytotoxicity test (MHC class I)
2. Mixed lymphocyte culture/reaction (MHC class II)
3. Primed lymphocyte typing
4. DNA analysis.

Classification

MHC gene product is classified based on their structure, cellular distribution, and function into **three groups** (Fig. 6.20). MHC class I and class II gene products are critical for immunologic specificity and transplantation histocompatibility, and they play a major role in susceptibility to a number of autoimmune diseases.

Class I MHC Molecules

- They are the products of MHC class I genes and are **expressed on all nucleated cells and platelets** (except erythrocytes and trophoblasts).

- They are **encoded by three closely linked loci, designated *HLA-A*, *HLA-B* and *HLA-C***.
- **Highly polymorphic** in the population and most highly polymorphic segment known within the human genome.
- **Functions:** Products of MHC class I gene are integral participants in the **immune response to intracellular infections, tumors and allografts**.
- Class I molecules **interact with CD8⁺ T lymphocytes during antigen presentation** and are involved in **cytotoxic reactions**. CD8⁺ T lymphocytes recognize antigens only in the context of self- class I molecules, they are referred to as class I MHC-restricted.

Class I MHC molecules:

1. Present in all nucleated cells and platelets
2. Not present in mature RBCs and trophoblasts.

Class II MHC Molecules

- They are encoded in a region called ***HLA-D***, which has three sub-regions: ***HLA-DP*, *HLA-DQ*, and *HLA-DR***.
- Class II antigens (*HLA-D* and *-DR*, D-related) are **expressed only on professional antigen-presenting cells** (B lymphocytes, monocytes/macrophages, Langerhans' cells, dendritic cells).
- **Function:** This locus contains genes that **encode many proteins involved in antigen processing and presentation**. The **class II-peptide complex is recognized by CD4⁺ T-cells** (function as helper cells) and these CD4 molecule acts as the co-receptor. Because **CD4⁺ T-cells can recognize antigens only in the context of self-class II molecules** → they are referred to as **class II MHC-restricted**.

Class II molecules are expressed only on professional antigen presenting cells.

Class III MHC Molecules

- Their gene **encode components of the complement system, cytokines, tumor necrosis factor (TNF)**,

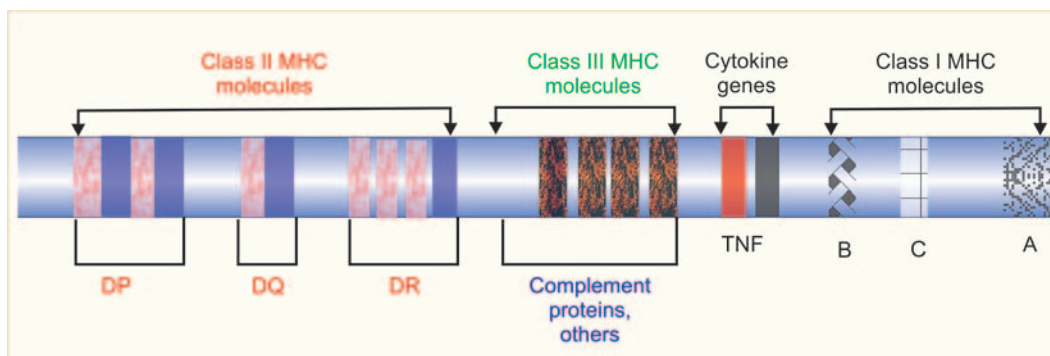


Fig. 6.20: Major histocompatibility complex (human leukocyte antigen complex) showing location of genes

lymphotoxin and some proteins without apparent role in the immune system.

MHC class III genes encode tumor necrosis factor.

Class III MHC gene code for:

1. Complement proteins (except C3)
2. Properdin B of alternate complement pathway
3. Tumor necrosis factor α and β .

HLA and Disease Association (Table 6.11)

Q. Write short note on diseases associated with HLA.

Some diseases are associated with the inheritance of certain HLA alleles and these diseases can be broadly grouped into:

- **Inflammatory diseases:** For example, **ankylosing spondylitis most strikingly associated with HLA-B27.**
- **Autoimmune diseases:** For example, autoimmune endocrinopathies associated with alleles at the DR locus.
- **Inherited errors of metabolism:** For example, 21-hydroxylase deficiency (HLA-BW47) and hereditary hemochromatosis (HLA-A).

HLA B27 is positive in ankylosing spondylitis.

Significance of HLA antigens:

1. Organ transplantation
2. Play major role in recognition of foreign antigen and immunity
3. Transfusion medicine
4. Its association with diseases.

REJECTION OF TRANSPLANTS

Q. Write short note on transplant rejection.

- Transplantation is a procedure for replacement of irreparably damaged tissue or organ to restore their lost function.
- Tissue or organ transplanted is called as **transplant** or **graft**.
- Individual from which transplant is obtained is known as **donor** and the individual who receives it is called **recipient**.
- **Allograft** is the term used for a **graft from individual of the same species**.
- A **major barrier for transplantation** is the process known as **rejection**, in which the recipient's immune system recognizes the graft as being foreign and mounts the immunological reactions against it.

ABO blood group compatibility: Most essential requirement for successful transplantation.

TABLE 6.11: Association with HLA alleles with diseases

Disease	HLA Allele
Class I MHC molecules	
• Ankylosing spondylitis	B27
• Postgonococcal arthritis	
• Acute anterior uveitis	
• Behçet's syndrome	B51
• 21-hydroxylase deficiency	HLA-A
• Hereditary hemochromatosis	HLA-BW47
Class II MHC molecules	
• Chronic active hepatitis	DR3
• Primary Sjögren syndrome	
• Rheumatoid arthritis	DR4
• Ulcerative colitis	DR103
• Type 1 diabetes	DR3/DR4
• Primary biliary cirrhosis	DR8
• Graves' disease and myasthenia gravis	DR3

Mechanism of Immune Recognition and Rejection of Allograft

- Transplantation rejection is a complex phenomenon and it is mainly due to antigenic differences between a donor and recipient's MHC molecules.
- Graft survives when MHC antigens of recipient closely matches with the donor.
- Both cell-mediated immunity and circulating antibodies play a role in transplant rejection.

T-cell Mediated Graft Rejection

- T-cells are the most important cells involved in allograft rejection.
- Host immune recognizes and responds to graft tissue by two pathways (Fig. 6.21).

Direct Recognition (Direct Pathway)

- **Direct recognition** is the **major pathway** in acute cellular rejection. During this pathway **MHC antigens on graft APCs are directly recognized by host CD8⁺ cytotoxic cells (class I MHC) and CD4⁺ helper T-cell (class II MHC)**, followed by their activation.

- **Consequences:**

- Killing of graft cells by CTLs:** Host $CD8^+$ T-cells which recognize class I MHC antigen on the APCs in the graft → differentiate into cytotoxic T-cells (CTLs) → kills parenchymal and endothelial cells in the graft tissue. The endothelial damage results in thrombosis and ischemia of graft tissue.
- Inflammatory reaction:** Host $CD4^+$ helper T-cells which recognize class II MHC antigens → proliferate → produce cytokines (e.g. $INF-\gamma$) → stimulate delayed type hypersensitivity inflammatory reaction (local accumulation of lymphocytes and macrophages) → damage to the graft. $CD4^+$ T-cells may also be activated by indirect pathway.

Indirect Recognition (Indirect Pathway)

- MHC molecules and antigen of the graft cell may be taken up and processed by the host's APCs (similar to other foreign antigens such as microbial antigens).
- Recognition of APCs with graft antigen by the host's $CD4^+$ T-cells → activates $CD4^+$ T-cells. This has two effects:
 - Stimulation of B lymphocytes** which transform into plasma cells and produce antibodies against graft alloantigens → mediate rejection through to a lesser extent. These alloantibodies bind to graft endothelium → causing endothelial damage → thrombosis and vascular injury.
 - Stimulation of delayed hypersensitivity reaction** in the tissue and blood vessel by producing cytokines (e.g. $INF-\gamma$) as mentioned under direct pathway.

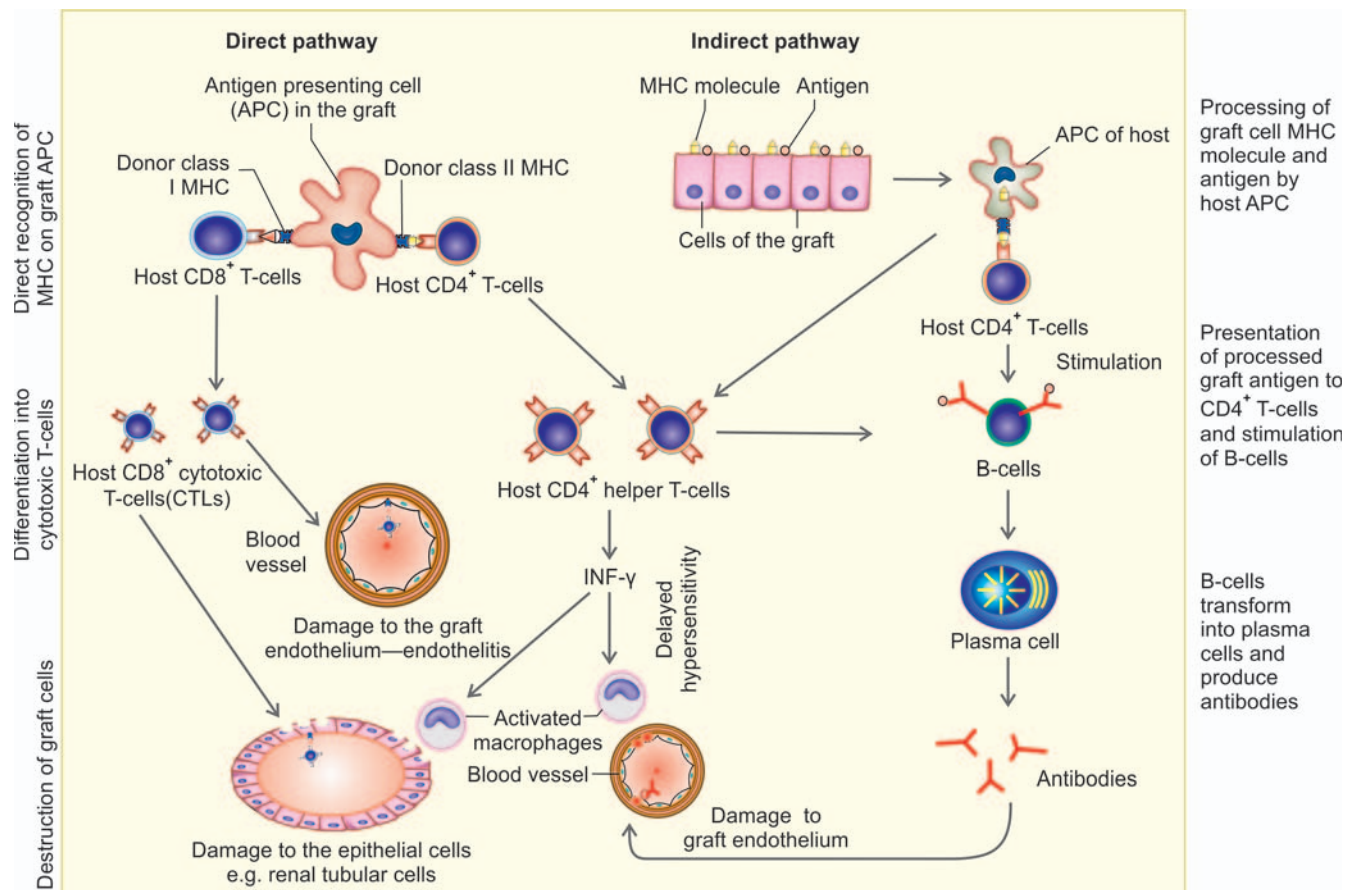


Fig. 6.21: Mechanism of recognition and rejection of allografts. There are two main pathways. In the direct pathway, donor MHC (class I and class II) antigens on antigen-presenting cells (APCs) in the graft are recognized by host $CD8^+$ cytotoxic T-cells and $CD4^+$ helper T-cells. $CD4^+$ cells produce cytokines (e.g. $INF-\gamma$) and damage graft cells by a delayed hypersensitivity reaction. $CD8^+$ T-cells differentiate into CTLs and kill graft cells. In the indirect pathway graft antigens are taken up, processed by host APCs, and presented to $CD4^+$ T-cells. This damages the graft by a local delayed hypersensitivity reaction and stimulates B lymphocytes to differentiate into plasma cells which produce antibodies

Antibody-mediated Graft Rejection

- T-cells play main role in the rejection of organ transplants. However, **antibodies produced against alloantigens** in the graft also mediate rejection and this is called **humoral rejection**.
- **Forms:** It can develop in two forms:
 - **Hyperacute rejection** (discussed below)
 - **Acute humoral rejection** sometimes referred to as rejection vasculitis (discussed below).

Graft rejection: Initiated by host T lymphocytes that recognize HLA antigen of graft as foreign.

Recognition of allograft may be direct (on APCs in the graft) or indirect (by host APCs).

Direct recognition: Important for acute graft rejection.

Indirect recognition: Important for chronic graft rejection.

Classification of Rejection Reaction (Table 6.12)

Q. Write short note on transplant rejection reactions.

Depending on time of occurrence, the rejection reactions are classified as: (1) **hyperacute**, (2) **acute** and (3) **chronic**.

Hyperacute Rejection

- Occurs within minutes or hours after transplantation
- It is a special type of rejection, occurs if the host has preformed anti-donor antibodies in the circulation before transplantation.

- These antibodies bind to endothelium of graft organ → **activates complement** → **vascular thrombosis**.
- Results in rapid and irreversible destruction of the graft.
- **Causes of preformed anti-donor antibodies:**
 - **Multiparous women**, who develop anti-HLA antibodies against paternal antigens that is shed from the fetus.
 - **Prior blood transfusions**, because platelets and white blood cells are rich in HLA antigens.
 - Host has **previously rejected a renal transplant**.

Hyperacute rejection: Caused by preformed antibodies.

Hyperacute rejection:

- Type II hypersensitivity reaction
- Irreversible.

Acute Rejection

- Occurs **within days to weeks** after transplantation in the non-immunosuppressed host
- Types:
 - **Acute cellular rejection:** It is mediated by activated T (CD4⁺ and CD8⁺) lymphocytes and results in deterioration in graft function.
 - **Acute humoral rejection (rejection vasculitis):** It is mediated by antibody (anti-donor antibodies) formed de novo after transplantation. Its consequences depend on specificity and ability to trigger other immune components such as the complement cascade.

Acute rejection:

- Most common
- Type IV and type II hypersensitivity.

TABLE 6.12: Classification and characteristics of transplant rejection

Type	Time	Mechanism	Pathological findings	
Hyperacute rejection	Minutes to hours	Preformed antibody and complement activation (type II hypersensitivity)	Arteritis, thrombosis and necrosis	Hyperacute rejection: Type II hypersensitivity.
Acute rejection				
• Acute cellular rejection	5 days to weeks	Activated T lymphocytes: CD4 ⁺ and CD8 ⁺ T-cells (type IV hypersensitivity)	Extensive interstitial mononuclear cell infiltration (CD4 ⁺ and CD8 ⁺), edema and endothelitis	Acute cellular rejection: Between 5 to 30 days of transplantation.
• Acute humoral rejection		Antibody and complement activation	Necrotizing vasculitis, neutrophilic infiltration and thrombosis	Acute rejection: <ul style="list-style-type: none"> • Type II hypersensitivity & • Type IV hypersensitivity.
Chronic rejection	Months to years	Immune and non-immune mechanisms	Fibrosis, scarring	Initial target of the antibodies in graft rejection is graft vasculature.

Acute humoral rejection: Antibodies destroy graft vessels.

Acute cellular rejection: T-cells destroy graft parenchymal cells and blood vessels by CTLs and inflammatory reaction.

Chronic Rejection

- Also known as **chronic allograft failure**.
- It is a major cause of graft loss.
- **Occurs months to years after transplantation.**
- Pathogenesis is poorly understood and may be due to both immunological and non-immunological mechanism.

Chronic rejection: Irreversible.

Corneal transplantation: Graft survival rate is very high.

Chronic rejection: Arteriosclerosis due to hyperplasia of vascular smooth muscle cells probably due to T-cell reaction and secretion of cytokines.

Q. Write short note on hyperacute rejection.

Q. Differences between hyperacute and acute transplant rejection.

MORPHOLOGY

Kidneys were the first solid organs to be transplanted and are more commonly transplanted organ; the morphologic changes are mainly in relation to renal transplants.

1. Hyperacute rejection:

- **Gross:**
 - The renal **graft rapidly becomes cyanotic, mottled, and flaccid**, and may excrete a few drops of bloody urine.
 - Later, cortex undergoes necrosis (infarction), and kidney becomes nonfunctional.
- **Microscopy:** **Blood vessels show widespread acute arteritis, arteriolitis with fibrinoid necrosis** of their walls → thrombosis → **ischemic necrosis**.

2. Acute rejection

- **Type:** Either cellular or humoral immune mechanisms may predominate.
 - **Acute cellular rejection:** It occurs within few months after transplantation and develops renal failure. It shows cellular infiltration of CD4⁺ and CD8⁺ T-cells and mononuclear cells. CD8⁺ T-cells may injure vascular endothelial cells, causing **endothelitis**.
 - **Acute humoral rejection** (rejection vasculitis): Main target of the antibodies is the graft vasculature → manifest as **vasculitis**, endothelial cell necrosis and neutrophilic infiltration. Caused by anti-donor antibodies.

3. **Chronic Rejection:** It is associated with proliferation of transplant vascular smooth muscle, interstitial fibrosis and scarring. It presents **progressive renal failure**. It is characterized by vascular changes, interstitial fibrosis and loss of kidney parenchyma.

Solid organs that are transplanted include liver, heart, lungs, and pancreas.

C4d Staining

- It is a fragment of complement protein C4.
- Its deposition in the capillaries of the graft indicates local activation of classic pathway of complement system and thereby provides an evidence for antibody-mediated damage.
- This is useful for the early detection of vascular rejection.

C4d staining: Useful in the early diagnosis of vascular rejection.

Transplantation of Hematopoietic Cells

Definition: Hematopoietic stem cell (HSC) transplantation is a procedure which involves eliminating an individual's hematopoietic and immune system by chemotherapy and/or radiotherapy and replacing with stem cells either from another individual or with individual's own hematopoietic stem cells.

Types of Hematopoietic Stem Cell Transplant

- Autologous ("from self"): Own HSCs removed, cryopreserved and re-infused.
- Allogeneic ("from different genes"): HSCs obtained from another individual.
- Syngeneic ("from same genes"): HSCs obtained from an identical twin.

Sources of Hematopoietic Stem Cells

- Bone marrow: Richest store.
- Peripheral blood: Very few HSCs but can be mobilized from bone marrow by administering G-CSF or GM-CSF.
- Umbilical cord blood: Easily available and is a rich source.

Complications of Hematopoietic Stem Cell Transplantation

Autologous HSC transplants have fewer immunologic complications but have higher rates of relapse of the disease after transplant. Allogeneic HSC transplants have lower

rates of relapse but have more immunologic complications, and GVHD, which can be fatal.

Graft Versus Host Disease (GVHD)

Q. Write short note on graft versus host disease.

It is the major complication that follows allogeneic HSC transplant. This is due to infused donor T lymphocytes (CD4⁺ and CD8⁺ T-cells) reacting against the recipient's tissues/organs. Three conditions are necessary for the development of GVHD:

- An immunocompetent graft (i.e. one containing T-cells).
- HLA mismatch (minor or major) between donor and recipient.
- An immunosuppressed recipient who cannot mount an immune response to the graft.

When immunosuppressed recipients receive normal bone marrow cells from allogeneic donors, the **immunocompetent T-cells present in the donor HSCs** recognize the recipient's **HLA antigens as foreign** and react against them.

- Acute GVH disease: It occurs before 100 days. It often affects three primary target organs simultaneously, namely skin, gastrointestinal (GI) tract and liver. Direct cytotoxicity by CD8⁺ T-cells, cytokines released by the sensitized donor T-cells is responsible for the damage.
- Chronic GVH disease: It occurs after day 100 and can affect the skin, GI tract, liver, eyes, lungs and joints. GVHD is difficult to treat and in severe cases it is usually fatal.

Target organs of GVH disease: Skin, gastrointestinal (GI) tract and liver.

Other Complications

- Infections:** Patients are susceptible to a variety of infections (bacterial, viral and fungal) due to lack of granulocytes, as well as lack of a functioning immune system.
- Organ toxicity:** Damage to GI tract, liver and lungs.
- Immunodeficiency:** It is a frequent complication of bone marrow transplantation. The immunodeficiency may be due to prior treatment, preparation for the graft, a delay in repopulation of the recipient's immune system, and attack on the host's immune cells by grafted lymphocytes. Immunodeficiency predisposes to infections, particularly infection with cytomegalovirus which can cause fatal pneumonitis.

IMMUNODEFICIENCY SYNDROMES

Immunodeficiency is defect in immunity.

Classification

- Primary immunodeficiency (PID)** disorders due to an intrinsic defect in the immune system.
- Secondary immunodeficiency** states which may arise as complications of an underlying condition. The underlying condition includes cancers, infections, malnutrition, or immunosuppression, irradiation, or chemotherapy for cancer and other diseases.

Primary Immunodeficiency

Classification of primary immune deficiency diseases is presented in Box 6.1. Most of them manifest themselves in infancy, between 6 months and 2 years of life. They come to clinical attention because they are susceptible to recurrent infections.

X-linked Agammaglobulinemia (Bruton's Agammaglobulinemia)

- One of the more common primary immunodeficiency disease.
- Characterized by **defect in B-cell development**.

Etiology

- Due to mutations in a cytoplasmic tyrosine kinase gene, called **Bruton tyrosine kinase (*Btk*) gene**. The gene is located on the long arm of the X chromosome at Xq21.22.
- Btk* gene product is a kinase that is required for maturation of pre B-cell to B-cell stage.
- Mutation of *Btk* gene blocks B-cell maturation at pre B-cell stage → no production of light chains** and reduced production of immunoglobulin. They have intact T-cell mediated immunity.

Clinical Manifestation

- Seen in **males** and does not manifest till about 6 months of age (till maternal immunoglobulins are depleted).
- Susceptible to infections:**
 - Recurrent bacterial infections of the respiratory tract, such as acute and chronic pharyngitis, sinusitis, otitis media, bronchitis and pneumonia.
 - Viral infections (e.g. echovirus, poliovirus and coxsackievirus) and *giardia lamblia*.
- Increased **susceptibility to autoimmune diseases** (e.g. arthritis and dermatomyositis).

Characteristic Findings

- Absent or markedly decreased B lymphocytes** in the circulation.

BOX 6.1: Classification of primary immune deficiency diseases*Deficiencies of the Innate Immune System*

- **Phagocytic cells:** E.g. impaired adhesion (leukocyte adhesion deficiency /LAD), impaired killing (chronic granulomatous disease /CGD)
- **Innate immunity receptors and signal transduction:** Defects in toll-like receptor signaling
- **Complement deficiencies:** Classical, alternative and lectin pathways.

Deficiencies of the Adaptive Immune System

- **T lymphocytes:** E.g. DiGeorge syndrome, Wiskott-Aldrich syndrome
- **B lymphocytes:** E.g. XL and AR agammaglobulinemia, hyper-IgM syndrome.

Regulatory Defects

- **Innate immunity:** E.g. severe colitis
- **Adaptive immunity:** E.g. autoimmune lymphoproliferation syndrome (ALPS).

- **Decreased serum levels of all classes of immunoglobulin.**
- **Underdeveloped germinal centers** in lymph nodes, Peyer's patches, the appendix and tonsils.
- **Absence of plasma cells throughout the body.**
- **Normal T-cell mediated immunity.**

Bruton disease: Usually does not manifest until about 6 months of age, when maternal immunoglobulin are depleted.

Bruton disease: Underdeveloped or rudimentary germinal centers in lymph nodes, Peyer's patches, the appendix and tonsils.

DiGeorge Syndrome (Thymic Hypoplasia)

- **T-cell immunodeficiency disorder:** Absence of cell-mediated immunity due to low numbers of T lymphocytes in the blood and lymphoid tissues.

Etiology

- **Defective embryologic development of the third and fourth pharyngeal pouches**, which normally give rise to the thymus, parathyroid glands, some of the clear cells of the thyroid, the ultimobranchial body and influence conotruncal cardiac development.
- Patients develop a **variable loss of T-cell mediated immunity** (due to hypoplasia or lack of the thymus), tetany (due to lack of the parathyroids) and congenital defects of the heart and great vessels (due to conotruncal cardiac development).

- In the absence of a thymus, T-cell maturation is interrupted at the pre T-cell stage.
- Most patients with DiGeorge syndrome have a point deletion (**22q11 deletion**) in the long arm of chromosome 22.

DiGeorge syndrome: Defective embryologic development of the third and fourth pharyngeal pouches.

DiGeorge syndrome: Absence of thymus and parathyroid glands.

Clinical Manifestations

- Usually presents during infancy with conotruncal congenital heart defects and severe hypocalcemia (due to hypoparathyroidism).
- Infants are prone to recurrent or chronic viral, bacterial, fungal and protozoal infections.
- The T-cell zones of lymphoid organs (paracortical areas of the lymph nodes and the periarteriolar sheaths of the spleen) are depleted.

Immunodeficiency with Thrombocytopenia and Eczema (Wiskott-Aldrich Syndrome)

- **X-linked recessive disease** characterized by thrombocytopenia, eczema and a marked susceptibility to recurrent infection, ending in early death.

Etiology

- Caused by **mutations in the WASP gene** encoding *Wiskott-Aldrich syndrome protein (WASP)*, which is located at Xp11.23 → reduced levels of WASP
- WASP link membrane receptors (e.g. antigen receptors) to cytoskeletal elements.
- WASP gene mutations affect not only T lymphocytes but also the other lymphocyte subsets, dendritic cells and platelets.

Clinical Manifestations

- Typically present with **recurrent bacterial infections, eczema and bleeding** caused by thrombocytopenia.

Other Features

- Thymus is morphologically normal.
- Later stages → progressive secondary depletion of T lymphocytes in the peripheral blood and in the T-cell zones (paracortical areas) of the lymph nodes, with variable loss of cellular immunity.
- Increased risk of developing non-Hodgkin B-cell lymphomas.

Wiskott Aldrich syndrome

- X-linked recessive
- Thrombocytopenia
- Eczema/atopic dermatitis
- Recurrent infections.

Wiskott Aldrich syndrome—diagnosis:

- Mutations in WASP gene at Xp11.23
- Reduced levels of WASP.

ACQUIRED IMMUNODEFICIENCY SYNDROME

Acquired immunodeficiency syndrome (AIDS) is caused by the retrovirus **human immunodeficiency virus (HIV)**.

Characteristic Features

- **Infection and depletion of CD4⁺ T lymphocytes.**
- **Severe immunosuppression → leads to opportunistic infections, secondary neoplasms and neurologic manifestations.**

AIDS: Commonest secondary immunodeficiency disorder.

Route of Transmission

Transmission of HIV occurs when there is an exchange of blood or body fluids containing the virus or virus-infected cells. The three major routes of transmission are:

1. **Sexual transmission:** It is the **main route of infection** in more than 75% of cases of HIV.
 - **Homosexual or bisexual men or heterosexual contacts:** It may be male-to-male, or male-to-female or female-to-male transmission.
 - **HIV is present in genital fluids** such as vaginal secretions and cervical cells (in women) and semen (in men).
 - Risk of sexual transmission of HIV is increased when there is coexisting sexually transmitted diseases, especially those associated with genital ulceration (e.g. syphilis, chancroid and herpes).
 - **Viral transmission** can occur in two ways:
 - **Direct inoculation of virus or infected cells into the blood vessels** at the site of breach caused by trauma, and
 - **By uptake into the mucosal dendritic cells (DCs).**

HIV: Sexual contact least efficacious, yet most common mode of spread.

HIV: Male-to-female transmission is more common compared to transmission from female-to-male.

2. **Parenteral transmission:** Three groups of individuals are at risk.
 - **Intravenous drug abusers:** Transmission occurs by **sharing of needles and syringes contaminated** with HIV-containing blood.
 - **Hemophiliacs:** Mainly those who received large amounts factor VIII and factor IX concentrates before 1985. Now increasing use of recombinant clotting factors have eliminated this mode of transmission.
 - **Transfusion of blood or blood components:** Recipients of blood transfusion of **HIV-infected whole blood or components** (e.g. platelets, plasma) was one of the modes of transmission. Screening of donor blood and plasma for antibody to HIV has reduced the risk of this mode of transmission. Because of recently infected individual may be antibody-negative (seronegative), there is a small risk of acquiring AIDS through transfusion of blood. Organs from HIV-infected donors can also transmit AIDS.

Risk of transmission by needle prick injury is 0.3% for HIV whereas for hepatitis it is 30%.

3. **Perinatal transmission (mother-to-infant transmission):**
 - **Major mode of transmission of AIDS in children.**
 - Transmission of infection can occur by **three routes**:
 - **In utero:** It is transmitted by transplacental spread.
 - **Perinatal spread:** During normal vaginal delivery or child birth (intrapartum) **through an infected birth canal** and in the immediate period (peripartum).
 - **After birth:** It is transmitted by ingestion of breast milk or from the genital secretions.

Transmission of HIV infection to health care workers: There is an extremely small risk of transmission to healthcare professional, after accidental needle-stick injury or exposure of nonintact skin to infected blood.

HIV: Neither transmitted by casual personal contact (in the household, workplace or school) nor by insect bites.

Vertical transmission: Commonest cause of AIDS in children.

Most common route for vertical transmission: Through infected birth canal during normal vaginal delivery.

Etiology

Properties of HIV

AIDS is caused by HIV, which is a **nontransforming human retrovirus** belonging to the lentivirus family. Retroviruses are **RNA viruses having an enzyme called reverse transcriptase**, which prepares a DNA copy of the RNA genome of the virus in host cell.

Genetic forms: HIV occurs in two genetically different but related main forms, **HIV-1 and HIV-2**.

- **HIV-1** is most common in the United States, Europe and Central Africa.
- **HIV-2** is common in West Africa and India.

Structure of HIV (Fig. 6.22)

Q. Write short note on structure of HIV.

- HIV-1 is **spherical enveloped virus** which is about 90–120 nm in diameter.
- It consists of electron-dense, cone-shaped core surrounded by nucleocapsid cell which is covered by lipoprotein envelope.

A. Viral core: It contains:

1. **Major capsid protein p24:** This viral antigen and the antibodies against this are **used for the diagnosis of HIV infection** in enzyme-linked immunosorbent assay (ELISA).
2. **Nucleocapsid protein p7/p9.**
3. **Two identical copies of single stranded RNA genome.**

4. **Three viral enzymes:** 1) **Protease**, 2) **reverse transcriptase** (RNA-dependent DNA polymerase), and 3) **integrase**. When the virus infects a cell, **viral RNA is not translated, instead transcribed by reverse transcriptase into DNA**. The **DNA form of the retroviral genome** is called a **provirus** which can be **integrated into the chromosome of host cell**.

Most common viral antigen used for diagnosis of HIV in blood before transfusion is p24.

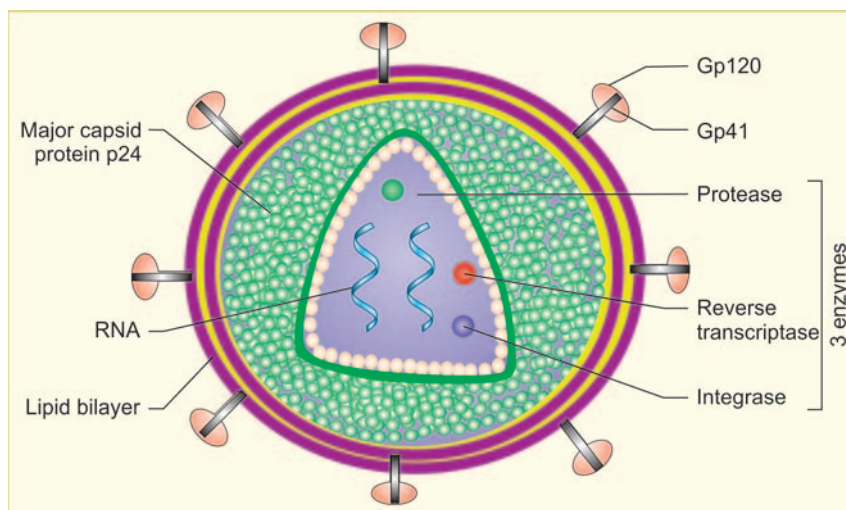
p24 antigen is a product of gag gene of HIV.

- B. Nucleocapsid:** The **viral core is surrounded by a matrix protein p24 and p17**, which lies **underneath the lipid envelope** of the virion.
- C. Lipid envelope:** The virus contains a lipoprotein envelope, which **consist of lipid derived from the host cell and two viral glycoproteins**. These glycoproteins are: 1) **gp120**, project as a knob-like spikes on the surface and 2) **gp41**, anchoring transmembrane pedicle. **These glycoproteins are essential for HIV infection of cells**.

HIV Genome

It contains two main groups of genes and their products act as antigens.

1. **Standard genes:** HIV-1 RNA genome **contains three standard retroviral genes**, which are **typical of retroviruses**. These include: **gag, pol, and env** genes. Initially, the protein products of the **gag and pol** genes are translated into **large precursor proteins** and are later



HIV: Anti-HIV-1 protease inhibitors inhibit formation of mature viral proteins, thereby preventing viral assembly.

Genomic variability: HIV-1 shows considerable variability in certain parts of their genome and is responsible for the difficulty in developing a single antigen vaccine against HIV.

CMV: Most common cause of blindness in AIDS patients.

Fig. 6.22: Diagrammatic representation of structure of the human immune deficiency virus (HIV)-1 virion. The viral particle is covered by a lipid bilayer derived from the host cell and studded with viral glycoproteins gp41 and gp120

cleaved by the viral enzyme protease to form → **mature proteins**.

2. **Accessory genes:** HIV contains accessory genes: E.g. tat, rev, vif, nef, vpr, and vpu. They **regulate the synthesis and assembly of infectious viral particles** and the **pathogenicity of the virus**.

Pathogenesis of HIV Infection and AIDS

Q. Write short note on pathogenesis of HIV infection and AIDS.

Infection is transmitted when the virus enters the blood or tissues of an individual.

Major targets: HIV can infect many tissues, but two major targets of HIV infection are:

- Immune system
- Central nervous system (CNS).

Life Cycle of HIV

Q. Write note on life cycle of HIV.

Consists of four main steps, namely: (1) Infection of cells by HIV, (2) integration of the provirus into the host cell genome, (3) activation of viral replication, and (4) production and release of infectious virus (Fig. 6.23).

1. Infection of cells by HIV:

- **Cell tropism:** HIV has **selective affinity** for host cells with **CD4 molecule receptor**. The cells with such receptors include **CD4⁺ T-cells** and other CD4⁺ cells such as **monocytes/macrophages** and **dendritic cells**. The HIV envelope contains two glycoproteins, surface

gp120 noncovalently attached to a transmembrane protein, gp41.

- **Gp120 of HIV binding to CD4 molecule receptor** on the host cell is the first step in HIV infection. Binding alone is not enough for infection and **requires participation of a coreceptor molecule**.
 - **Conformational change:** Binding to CD4 leads to a conformational change in the HIV, that results in the formation of a new recognition site on gp120 for the coreceptors CCR5 or CXCR4.
 - **Gp120 binding to chemokine receptor:** New recognition site on gp120 of HIV bind to **chemokine receptors**, i.e. CCR5 and CXCR4.
 - **Penetration of host cell membrane by gp41:** Binding of gp120 to the chemokine coreceptors leads to **conformational changes in gp41**.
 - **Membrane fusion:** The conformational change in gp41 allows **HIV to penetrate the cell membrane of the target cells** (e.g. CD4⁺ T-cells or macrophages), leading to **fusion of the virus with the host cell**.
 - **Entry of viral genome into cytoplasm of host cell:** Once internalized, the virus core containing the HIV genome enters the cytoplasm of the host cell.
2. **Integration of the proviral DNA into the genome of the host cell:**
 - After the internalization of the virus core, the **RNA genome of the virus undergoes reverse transcription** → leading to the **synthesis of double-stranded complementary DNA (cDNA/proviral DNA)**.
 - **Episomal form:** In quiescent T-cells, HIV cDNA may remain as a linear **episomal form** in the cytoplasm of infected cell.

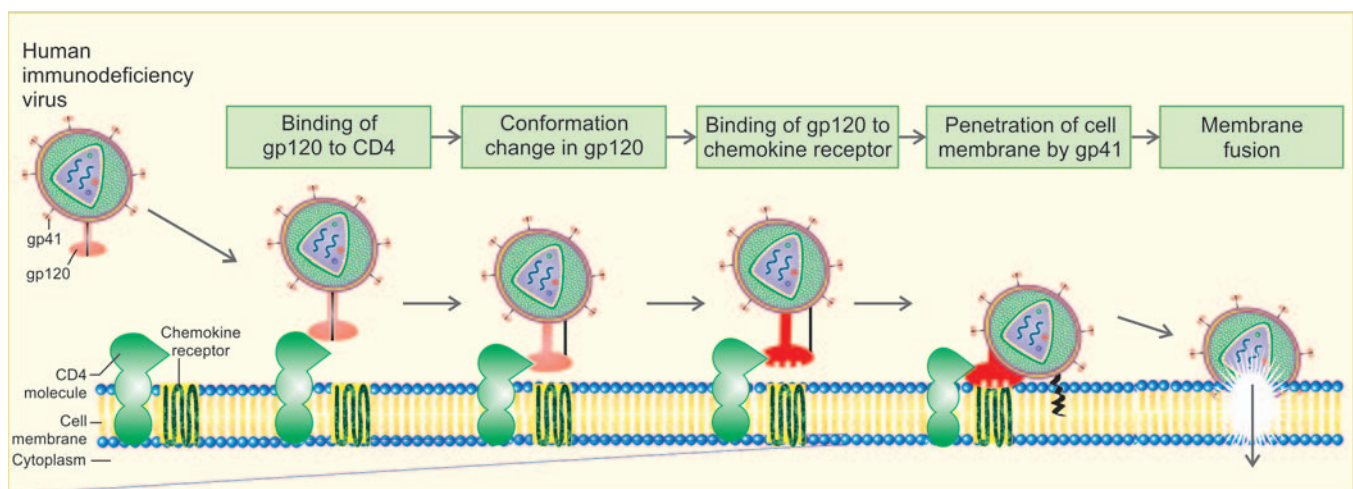


Fig. 6.23: Various molecular steps involved in the life cycle of HIV

- **Integration of cDNA:** In dividing T-cells, HIV cDNA enters the nucleus, and becomes integrated into the genome of the host cell using a viral integrase protein.
3. **Viral replication:** After the integration of proviral DNA, it can either be latent or productive infection.
 - **Latent infection:** During this, the provirus remains silent for months or years.
 - **Productive infection:** In this, the proviral DNA is transcribed → leading to viral replication → formation of complete viral particles.
 4. **Production and release of infectious virus:** The complete virus particle formed, buds from the cell membrane and release new infectious virus. This productive infection when extensive, leads to death of infected host cells.

The virus infection remains latent for long periods in lymphoid tissues. Active viral replication is associated with more infection of cells and progression to AIDS.

Dissemination: Virus disseminates to other target cells. This occurs either by fusion of an infected cell with an uninfected one or by the budding of virions from the membrane of the infected cell.

HIV: Selective affinity for host cells with CD4 molecule receptor and includes:

1. CD 4⁺ T-cells (worst affected)
2. Monocytes/macrophages
3. Dendritic cells.

Defective CCR5 receptors lead to protective effect of providing resistance to the development of AIDS.

Neutrophil is not a target for initiation and maintenance of HIV infection.

Progression of HIV Infection

Acute Infection

HIV infection starts as an acute infection. It is only partially controlled by the host immune response and progresses to chronic infection of peripheral lymphoid tissue.

- **Primary infection:** HIV first infects memory CD4⁺ T-cells (express CCR5), which are present in the mucosal lymphoid tissue (largest reservoir of T-cells and where majority of memory cells are lodged). HIV causes death of these cells resulting in significant depletion of T-cells.
- **Spread to lymphoid tissue:** Dendritic cells at the primary site of infection capture the virus and migrate to lymphoid tissue such as lymph nodes and spleen. In

the lymphoid tissues, DCs are passed on to CD4⁺ T-cells by direct cell-to-cell contact.

- **Acute HIV (retroviral) syndrome:** Virus replicates and causes viremia, accompanied by acute HIV syndrome (nonspecific signs and symptoms similar to many viral diseases). The extent of viremia is measured as HIV-1 RNA levels in the blood. It is a useful marker of HIV disease progression and in the management of HIV infection.
- **Host immune response against HIV:** Virus spreads throughout the body and infects helper T-cells, macrophages and DCs in the peripheral lymphoid tissues. During this period, the host humoral and cell-mediated immune response develops against viral antigens. These include anti-HIV antibodies and HIV-specific cytotoxic T-cells. Immune responses partially control the infection and viral replication.

Chronic Infection: Clinical Latency Period

Following acute phase it progress to chronic phase. This phase is characterized by dissemination of virus, viremia, and development of immune response by host.

- **Minimal/no symptoms:** In this phase, virus continuously replicates in the lymph nodes and spleen. The host immune response can handle most infections with opportunistic microbes with no or minimal clinical symptoms.
- **Progressive decrease of CD4⁺ T-cells:** There is continuous destruction of CD4⁺ T-cells in the lymphoid tissue accompanied by steady decrease in their number in the peripheral blood. During the early course of disease, the loss of CD4⁺ T-cells can be replaced by new T-cells. However, over a period of years, the continuous cycle of viral infection and death of T-cells → leads to steady decrease in the number of CD4⁺ T-cells both in the lymphoid tissue and in circulation.

Mechanism of T-cell depletion: Direct killing of T-cells by the virus is the major cause.

Inversion of CD4⁺/CD8⁺ ratio: Normal CD4⁺/CD8⁺ ratio is 1:2. Loss of CD4⁺ cells in AIDS patient leads to inversion of ratio of 0.5 or less.

HIV infection of non T-cells: HIV can infect non T-cells such as macrophages and dendritic cells (mucosal and follicular).

HIV is cytotoxic to CD4⁺ T-cells and leads to loss of cell-mediated immunity.

HIV affects most commonly: CD 4⁺ T (helper) cells.

Normal ratio of CD4 to CD8 is 2:1.

Abnormalities of B-cell Function

- Polyclonal activation of B-cells → hypergammaglobulinemia → circulating immune complexes.
- Impaired humoral immunity → disseminated infections caused by capsulated bacteria, such as *S. pneumoniae* and *H. influenzae*.

Natural History of HIV Infection (Fig. 6.24)

Q. Write short note on natural history of HIV infection.

Virus usually enters the body through mucosal epithelia and clinical course can be divided into three main phases:

1. **Early acute phase:** It may present as an acute (refer above), usually **self-limited nonspecific illness**. These symptoms include sore throat, myalgias, fever, weight loss and fatigue. Other features, such as rash, cervical adenopathy, diarrhea and vomiting, may also occur.

Q. Write short note on CD count in HIV.

Centers for disease control (CDC) classification of HIV infection: It depends on blood CD4⁺ T-cell count. This divides the patients into three categories with counts being:

- greater than 500 cells/ μ L
 - between 200 and 500 cells/ μ L
 - less than 200 cells/ μ L
2. **Middle chronic phase:** It may have few or no clinical manifestations and is called the clinical latency period (refer page 150). The symptoms may be due to minor opportunistic infections, such as oral candidiasis (thrush), vaginal candidiasis, herpes zoster, and perhaps mycobacterial tuberculosis.
 3. **Final crisis phase:** It is final phase of HIV with progression to AIDS. It presents with fever, weight loss, diarrhea, generalized lymphadenopathy, multiple opportunistic infections, neurologic disease and secondary neoplasms. Most of untreated (but not all) patients with HIV infection progress to AIDS after a chronic phase lasting from 7 to 10 years.

Exceptions

- **Rapid progressors:** In these patients, the middle, chronic phase is shortened to 2–3 years after primary infection and they rapidly progress to AIDS.
- **Long-term nonprogressors:** It is defined as untreated patients who are asymptomatic for 10 years or more, with stable CD4⁺ T-cell counts and low levels of plasma viremia.

The opportunistic infections and neoplasms found in patients with HIV infection are presented in Table 6.13.

CNS lesions in AIDS

Q. List the CNS lesions found in AIDS.

- AIDS-dementia complex
- Non-Hodgkin B-cell lymphoma—primary lymphoma of the brain
- Progressive multifocal leukoencephalopathy
- Meningoencephalitis (tuberculous, cryptococcal)
- Aseptic meningitis
- Peripheral neuropathy
- Demyelinating lesions of the spinal cord

CNS in AIDS:

- Perivascular giant cell
- Vacuolization
- Microglial nodule.

Diagnosis of HIV Infection or AIDS

Q. Write short note on laboratory diagnosis of AIDS.

1. **ELISA:** Detects antibodies against viral proteins. It is the **most sensitive** and best screening test for the diagnosis of AIDS.
2. **Western blot:** Most specific or the **confirmatory test** for HIV.
3. **Direct detection of viral infection:**
 - p24 antigen capture assay.
 - Reverse transcriptase polymerase chain reaction (RT-PCR).
 - DNA-PCR.
 - Culture of virus from the monocytes and CD4⁺T-cells.

Prognosis: The prognosis of AIDS is poor.

Anti-gp120: Detected by ELISA test.

AMYLOIDOSIS

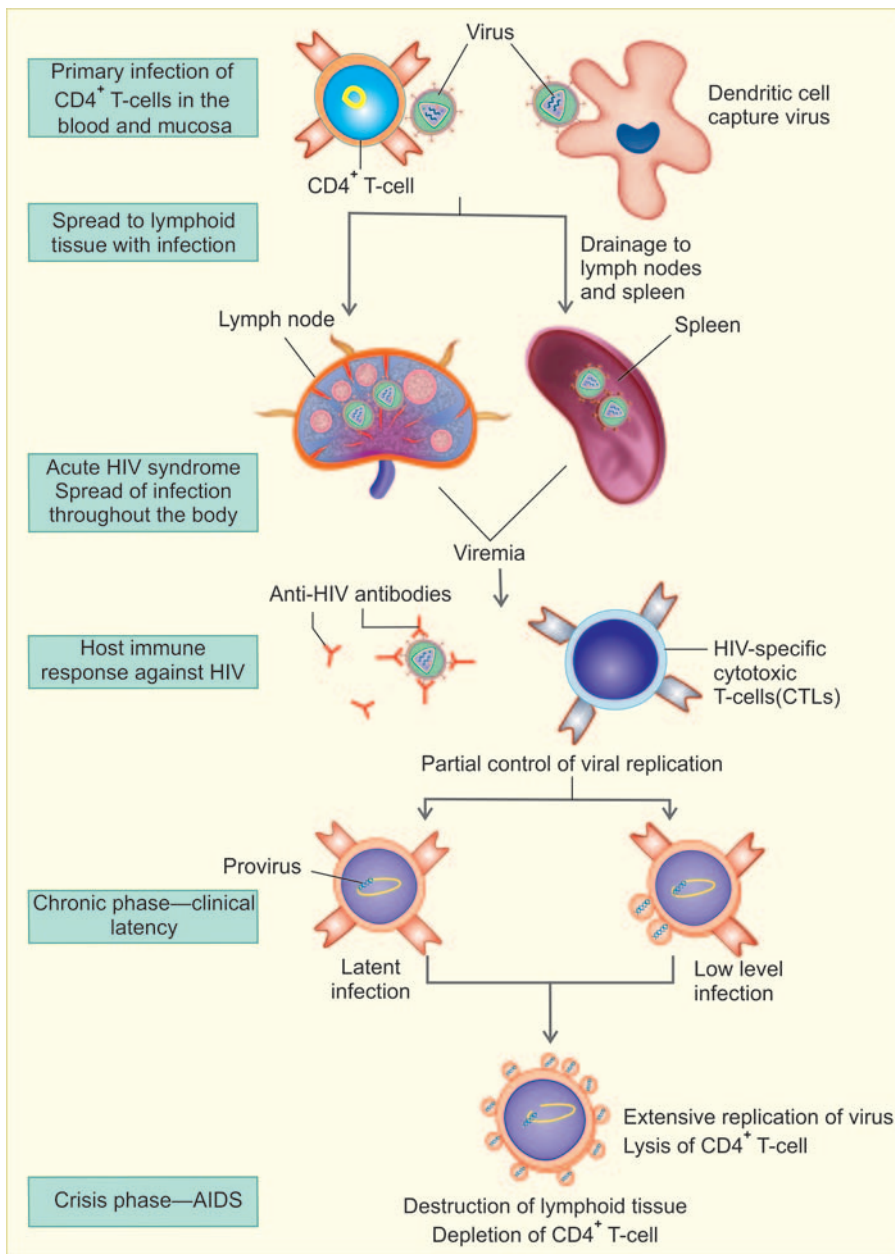
Q. Define amyloidosis.

Definition: Amyloid is a pathologic fibrillar protein deposited in the extracellular space in various tissues and organs of the body in variety of clinical condition.

Amyloidosis is characterized by extracellular deposition of misfolded proteins that aggregate to form insoluble fibrils.

General Features

- Associated with number of inherited and inflammatory disorders.



Natural history of HIV infection:

1. Early acute phase
2. Middle chronic phase
3. Final crisis phase.

Western blot: Confirmatory test for HIV.

CMV: Most common cause of blindness in AIDS patients.

Fig. 6.24: Pathogenesis of HIV infection. HIV infects CD4⁺ T-cells and dendritic cells, and spreads to lymph nodes. Viral replication in lymph node leads to viremia and widespread seeding of lymphoid tissue. The viremia is controlled by the host immune response and the disease enters a phase of clinical latency. During this phase, viral replication in both T-cells and macrophages continues. Ultimately, there is progressive decrease of CD4⁺ cells and patient develops clinical symptoms of full-blown AIDS entering the crisis phase

- **Extracellular deposits** cause structural and functional damage to involved tissue.
- **Basically a disorder of protein misfolding** and is produced by aggregation of misfolded proteins (normal folded proteins are soluble) or protein fragments.
- It also contains abundant charged sugar groups and has staining characteristics that were thought to resemble starch (amylose) and were called as amyloid. But these deposits are not related to starch.
- **Usually a systemic** (sometimes localized) disease.

Q. Write briefly on opportunistic infections in AIDS.

Q. Write briefly on common neoplasms in HIV patients.

TABLE 6.13: AIDS-defining opportunistic infections and neoplasms found in patients with HIV infection

Opportunistic infections	
Protozoal and Helminthic Infections	Organ or Site Involved or Type of Damage
Cryptosporidiosis or isosporidiosis	Enteritis
Toxoplasmosis	Pneumonia or CNS infection
Fungal Infections	
Pneumocystosis	Pneumonia or disseminated infection
Candidiasis	Esophageal, tracheal, or pulmonary
Cryptococcosis	Infection of central nervous system
Coccidioidomycosis	Disseminated
Histoplasmosis	Disseminated
Bacterial Infections	
Mycobacteriosis	
• Atypical, e.g. <i>Mycobacterium avium-intracellulare</i>	Disseminated or extrapulmonary
• <i>M. tuberculosis</i>	Pulmonary or extrapulmonary
Nocardiosis	Pneumonia, meningitis, disseminated
<i>Salmonella</i> infections	Disseminated
Viral Infections	
Cytomegalovirus	Pulmonary, intestinal, retinitis, or CNS infections
Herpes simplex virus	Localized or disseminated
Varicella-zoster virus	Localized or disseminated
Progressive multifocal leukoencephalopathy	Central nervous system
Neoplasms	Cause
Kaposi sarcoma (KS)	Kaposi sarcoma herpes virus
Non-Hodgkin B-cell lymphoma—primary lymphoma of the brain	Epstein Barr virus (EBV)
Cervical cancer in women	Human papilloma virus (HPV)
Anal carcinoma	HPV

Pneumocystis jiroveci:
Most common fungal infection in AIDS in World.

Candidiasis: Most common fungal infection in AIDS in India.

Most common vascular tumor in AIDS patients is:
Kaposi's sarcoma.

M. tuberculosis: Most common infection with HIV in India.

Most common site for lymphoma in AIDS patients is CNS.

AIDS: Malignancy includes Kaposi sarcoma (most common), NHL cervical and anal cancer.

AIDS: Death is usually due to disseminated infection.

Forms of Amyloid

All amyloid have same morphological and staining property but amyloidosis is not a single disease. It is a group of diseases having in common the deposition of similar-appearing proteins in which biochemical structure (more than 20 different proteins) and mechanism of formation are different.

Physical Nature of Amyloid

All types of amyloid are composed of nonbranching fibrils of 7–10 nm diameter.

- Each fibril consists of β -pleated sheet polypeptide chains and is wound around one another.
- Congo red dye binds to these fibrils and produces classic apple-green birefringence (dichromism).

Hence, Congo red stain is used to identify amyloid deposits in tissues.

X-ray crystallography and infrared spectroscopy shows characteristic cross β -pleated sheet configuration.

Electron microscopy of amyloid: Nonbranching fibrils of indefinite length and 7–10 nm diameter.

Chemical Nature of Amyloid

Q. Write short note on physical and chemical nature of amyloid.

Fibrillar proteins bind with variety of substances:

- **About 95%** of the amyloid material consists of **fibril proteins**.
- **Remaining 5%** consists of **proteoglycans, glycosaminoglycans, serum amyloid P, etc.**

Biochemical Forms of Amyloid

It consists of three major distinct proteins and more than 20 minor forms.

A. Major forms

These are AL, AA and A β amyloid—

1. **AL (amyloid light chain) protein:**
 - Consists of **complete immunoglobulin (Ig) light chains** or the **amino-terminal fragments of light chains, or both**.
 - **Produced by plasma cells** and associated with some monoclonal B-cell proliferation (e.g. plasma cell tumors).
2. **AA (amyloid-associated) protein:**
 - **Non-immunoglobulin**.
 - **Derived from** a larger precursor in the serum called **SAA (serum amyloid-associated) protein synthesized by the liver**. **Increased synthesis of SAA** protein occurs under the influence of cytokines (e.g. IL-6 and IL-1) **during inflammation**.
 - Associated with **chronic inflammation** (called as secondary amyloidosis).

Primary amyloidosis: B-cell neoplasm—AL type.

Secondary amyloidosis: Chronic inflammation—AA type.

3. **A β amyloid:**
 - **Derived from** transmembrane glycoprotein called **amyloid precursor protein (APP)**.
 - **Found in the cerebral lesions of Alzheimer disease**.

A β amyloid is found in association with Alzheimer disease.

B. Minor types

1. **Transthyretin (TTR):**
 - **It is a normal serum protein** that transports thyroxine and retinol.
 - **Mutations** in gene encoding TTR \rightarrow **alter its structure** \rightarrow misfolds.
 - **Found in a familial amyloid polyneuropathies, heart of aged individuals** (senile systemic amyloidosis).
2. **β_2 -microglobulin:**
 - It is a **normal serum protein**.
 - Amyloid fibril subunit namely A β_2 m is **derived from β_2 -microglobulin** and is found in amyloidosis of patients on **long-term hemodialysis**.
3. **Other minor types:** Serum amyloid P component, proteoglycans, and highly sulfated glycosaminoglycans.

Pathogenesis of Amyloidosis (Fig. 6.25)

Misfolding of Proteins

Q. Write short note on pathogenesis of amyloidosis.

Amyloidosis is a disorder due to abnormal folding or misfolding of proteins.

- **Normally, misfolded proteins are degraded either intracellularly** in proteasomes, or **extracellularly** by macrophages.
- **In amyloidosis, there is failure of control mechanism** \rightarrow production of misfolded proteins, which exceeds the degradation \rightarrow accumulation outside cells. These misfolded proteins are unstable and self-associated \rightarrow deposited as fibrils in extracellular tissues.

Categories of Proteins

Misfolded proteins that form amyloid may be the result of:

Production of Abnormal Amounts of Normal Protein

- These proteins have an inherent tendency to fold improperly or undergo misfolding \rightarrow associate and form fibrils. Example: During inflammation, SAA is synthesized by the liver cells under the influence of cytokines such as IL-6 and IL-1 secreted by activated macrophages, and is degraded by monocyte-derived enzymes.
- In individuals prone to amyloidosis, there may be defect in the monocyte-derived enzymes \rightarrow incomplete breakdown of SAA \rightarrow formation of insoluble AA molecules. Genetically defective SAA may also be responsible for resistant degradation by macrophages.

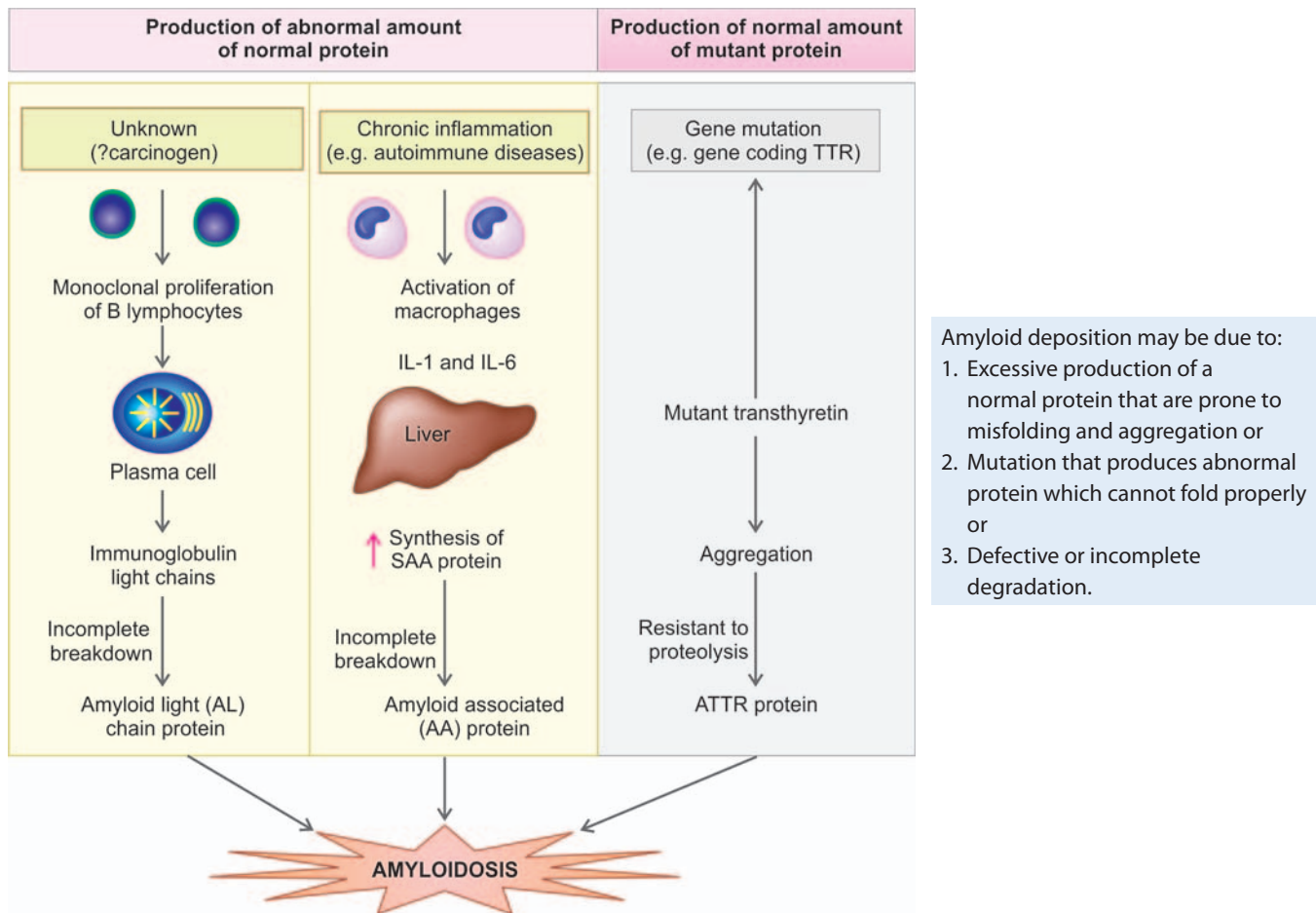


Fig. 6.25: Pathogenesis of amyloidosis. AL protein is seen in association with B lymphocyte and plasma cell proliferation which secrete immunoglobulin light chains that are amyloidogenic. AA protein is seen in variety of diseases associated with the activation of macrophages, which in turn leads to the synthesis and release of SAA. The SAA is converted to AA protein. ATTR protein is due to mutant proteins which aggregate and deposit as amyloid

Abbreviation: SAA, serum amyloid; ATTR, transthyretin.

Production of Normal Amount of Mutant Protein

This is the protein that is prone to misfolding and subsequent aggregation to form amyloid.

Example: In familial amyloidosis, mutation of gene encoding TTR → alterations in structure of serum protein TTRs → proteins prone to misfolding → aggregate → are resistant to proteolysis.

Pathological Effects

- **Pressure on adjacent normal cells** → leads to **atrophy of cells**.
- **Deposition in the blood vessel wall** causes:
 - Narrowing of the lumen → lead to **ischemic damage**.
 - **Increased permeability** → escape of protein out of vessel.

Amyloid causes pressure on adjacent cells and may lead to atrophy of cells as well as impair normal function of cells.

Classification of Amyloidosis

Q. Describe the pathology of primary amyloidosis.

Q. Classify amyloidosis.

Amyloidosis is classified **depending on biochemical and clinical characteristics** (Table 6.14).

Amyloidosis may be classified as systemic, hereditary or localized.

Systemic (Generalized)

It involves several organ systems.

Q. Describe the pathology of primary amyloidosis.

Primary Amyloidosis:

a. **Immunocyte dyscrasias with amyloidosis:** Usually systemic and is of AL type. Many have underlying plasma cell dyscrasia, e.g. multiple myeloma.

- **Multiple myeloma:**
 - 5–15% of patients develop amyloidosis.
 - Tumor synthesizes abnormal amounts of a single specific Ig (monoclonal gammopathy) → appears as an M (myeloma) protein spike on serum electrophoresis.
 - Tumor also synthesizes the light chains (known as Bence-Jones protein) of either the κ or the λ type which are found in the serum.
 - Bence-Jones protein being of small molecular size can be excreted in the urine. The amyloid deposits in these patients contain the same light chain protein.
 - All the myeloma patients with amyloidosis invariably have Bence-Jones proteins in the serum or urine, or both. But majority of myeloma patients who have free light chains do not develop amyloidosis. This suggests that the presence of Bence-Jones proteins, though necessary, is by itself not enough for amyloidosis.

b. **Primary amyloidosis without plasma cell dyscrasia:**

- Majority of patients with AL amyloid do not have multiple myeloma or any other overt plasma cell neoplasm.
- But almost all these patients have monoclonal immunoglobulins or free light chains, or both, in the serum or urine.
- Bone marrow in most show increase in the number of plasma cells, which may secrete the precursors of AL protein. Thus, these may represent plasma cell dyscrasia characterized by production of an abnormal protein, instead of production of tumor masses.

Bone marrow in AL amyloidosis shows: Plasmacytosis.

Reactive Systemic (Secondary) Amyloidosis

Q. Write short note on reactive systemic amyloidosis.

- Systemic in distribution and is of AA type.
- Occurs as a complication of (secondary to) chronic inflammatory or tissue-destructive process. Hence, was known as secondary amyloidosis.
- Complicates or occurs in association with diseases, such as:
 - Chronic inflammatory conditions: E.g. tuberculosis, bronchiectasis and chronic osteomyelitis.

TABLE 6.14: Classification of amyloidosis

Type	Precursor protein	Fibril protein	Associated disease/s
A. Systemic (generalized) amyloidosis			
1. Immunocyte dyscrasias with amyloidosis (primary amyloidosis)	Immunoglobulin light chains (mainly λ)	AL	Multiple myeloma, other plasma cell dyscrasias
2. Reactive systemic amyloidosis (secondary amyloidosis)	Serum amyloid associated (SAA)	AA	Chronic inflammatory process
3. Hemodialysis-associated amyloidosis	β_2 -microglobulin	A β_2 m	Chronic renal failure
B. Hereditary or familial amyloidosis			
1. Familial Mediterranean fever	SAA	AA	
2. Familial amyloidotic neuropathies	Transthyretin	ATTR	
3. Systemic senile amyloidosis			
C. Localized amyloidosis			
1. Senile cerebral	Amyloid precursor protein (APP)	A β	Alzheimer disease
2. Endocrine			
• Thyroid	Calcitonin	A Cal	Medullary carcinoma
• Islets of Langerhans	Islet amyloid peptide	AIAPP	Type 2 diabetes

- **Autoimmune states:** E.g. rheumatoid arthritis, ankylosing spondylitis, inflammatory bowel disease (Crohn's disease and ulcerative colitis).
- **Heroin abusers:** These patients develop with chronic skin infections or abscesses due to subcutaneous self-administration of narcotics.
- **Non-immunocyte-derived tumors:** E.g. renal cell carcinoma and Hodgkin lymphoma.

Secondary amyloidosis:

1. Associated with chronic inflammatory disorders
2. Amyloid is of AA type
3. Derived from acute phase protein SAA.

Hemodialysis-associated Amyloidosis

Patients with **chronic renal failure on long-term hemodialysis** have high levels of β_2 -microglobulin in the serum because it cannot be filtered through dialysis membranes → gets deposited as amyloid.

Hemodialysis associated carpal tunnel syndrome is associated with β_2 microglobulin.

Hereditary or Familial Amyloidosis

Q. Write short note on hereditary familial amyloidosis.

It constitutes a heterogeneous group, are rare and occur in certain geographic areas.

Familial Mediterranean Fever

- **Autosomal recessive** disorder.
- Characterized by recurrent attacks of fever accompanied with inflammation of serosal surfaces (peritoneum, pleura and synovial membrane).
- The gene encodes a protein called **pyrin** (for its relation to fever), regulate inflammatory reactions by producing high levels of pro-inflammatory cytokines IL-1.
- The amyloid fibril proteins are of AA type probably produced due to recurrent bouts of inflammation.

Familial Amyloidotic Neuropathies

It is characterized by **deposition of amyloid in peripheral and autonomic nerves** and the **fibrils are made up of mutant TTRs**.

Familial amyloidotic polyneuropathy is due to amyloidosis of nerves caused by deposition of: Mutant transthyretin (TTR).

Localized Amyloidosis

Q. Write short note on localized amyloidosis.

- Amyloid deposits are **limited to a single organ** (e.g. heart) or tissue.
- Either grossly visible as nodular masses or detected only by microscopic examination.
- **Sites:** Lung, larynx, skin, urinary bladder and tongue.
- **Microscopy:** Amyloid deposits may be surrounded by lymphocytes and plasma cells.

Endocrine Amyloid

Q. Write short note on endocrine tumors showing microscopic deposits of amyloid.

- Endocrine tumors such as **medullary carcinoma of the thyroid** (refer chapter 25 and Fig 25.13), islet tumors of

the pancreas, pheochromocytomas and undifferentiated carcinomas of the stomach.

- **Islets of Langerhans in type II diabetes mellitus.**

Medullary carcinoma of thyroid:

- Calcitonin is the tumor marker
- Calcitonin form amyloid ACal.

Amyloid of Aging

Q. Write briefly on amyloid of aging.

- **Senile systemic amyloidosis** characterized by the systemic deposition of amyloid in elderly patients usually between 70–80 years. Also called senile cardiac amyloidosis because of the symptoms related to restrictive cardiomyopathy and arrhythmias. **The amyloid is composed of the normal TTR molecule.**

MORPHOLOGY

Main Organs Involved

- **Secondary amyloidosis:** Kidneys, liver, spleen, lymph nodes, adrenals and thyroid.
- **Primary amyloidosis:** Heart, GI tract, respiratory tract, peripheral nerves, skin and tongue.

Gross

- May or may not be apparent grossly.
- If large amount accumulates → affected organs are **enlarged, firm and have a waxy appearance** (Fig. 6.26).
- **Cut surface:** If the amyloid deposits are large, **painting** the cut surface with **iodine** gives a **yellow color, which is transformed to blue violet after application of sulfuric acid** (which acidifies iodine). This method was used for demonstrating cellulose or starch. This staining property was responsible for the coining of the term **amyloid (starch-like)**. But it is neither starch nor cellulose.

Microscopy

Q. Write short note on special stains for amyloid.

Hematoxylin and Eosin Stain

- Amyloid deposits are always **extracellular** (Fig. 6.27 A) and begin between the cells. In AL form perivascular and vascular deposits are common.
- Progressive accumulation of **amyloid produces pressure atrophy of adjacent cells**.
- Appears as an **amorphous, eosinophilic, hyaline, glassy, extracellular substance**.
- Many other substances (e.g. collagen, fibrin) also stain eosinophilic with hematoxylin and eosin. Hence, it is necessary to differentiate amyloid from these other hyaline deposits by using special stains.

Staining (Tinctorial) Properties of Amyloid

1. **Congo red stain:** It is the special stain used for the diagnosis of amyloidosis. Amyloid stains pink or red with the Congo red dye under ordinary light (Fig. 6.27 B). But more specific when viewed under polarizing microscope; amyloid gives apple-green birefringence (Fig. 6.27 C). This reaction is due to the cross- β -pleated configuration of amyloid fibrils. Can be confirmed by electron microscopy.
2. **Van Gieson stains:** It takes up khaki color.
3. **Alcian blue:** It imparts blue color to glycosaminoglycans in amyloid.
4. **Periodic acid Schiff reaction (PAS):** It stains pink.
5. **Methyl violet and cresyl violet:** These metachromatic stains give rose pink color.
6. **Thioflavin T:** It is not specific for amyloid, but amyloid fluoresce when viewed in ultraviolet light.
7. **Immunohistochemical staining:** It can distinguish AA, AL and ATTR types.

Congo red stain: Amyloid gives apple-green birefringence when viewed under polarizing microscope.

MORPHOLOGY OF MAJOR ORGANS INVOLVED

Q. Describe the gross and microscopic features of organs involved in primary/secondary amyloidosis.

Kidney

Kidney involvement is the **most common** and the **most serious** form of organ involvement.

- **Gross:** It may be of normal size and color during early stages. In advanced stages, it may be shrunken due to ischemia, which is caused by vascular narrowing induced by the amyloid deposit within arterial and arteriolar walls.
- **Microscopy:** Most commonly renal amyloid is of light-chain (AL) or AA type.
 - **Glomeruli:** It is the main site of amyloid deposition (Fig. 6.28).
 - ◆ First, focal deposits within **mesangial matrix**, accompanied by diffuse or nodular thickening of the **glomerular basement membranes**.
 - ◆ Later, both the mesangial and basement membranes deposits cause **capillary narrowing**. Progressive accumulation of amyloid results in **obliteration of the capillary lumen** and glomerulus shows broad ribbons of amyloid.
 - Amyloid may also be deposited in the **peritubular interstitial tissue**, arteries and arterioles.

Spleen

Q. Describe the gross and microscopic appearance of spleen in amyloid (Sago and Lardaceous spleen).

- **Gross:** It may be normal in size or may cause moderate to marked splenomegaly (200–800 g). It may show one of two patterns of deposition.

- **Sago spleen:** Amyloid deposits are limited to the splenic follicles, which grossly appear like tapioca/sago granule; hence known as **sago spleen**. Microscopically, the amyloid is deposited in the wall of arterioles in the white pulp.
- **Lardaceous spleen:** Amyloid is deposited in the walls of the splenic sinuses and connective tissue framework in the red pulp. This may result in moderate to marked enlargement of spleen. Fusion of the early deposits give rise to large, map-like areas of reddish color on cut surface. This resembles pig fat (lardaceous) and hence called as **lardaceous spleen**. Microscopically, it shows amyloid deposits in the wall of the sinuses.
- **Light microscopy:** These deposits appear homogenous pink, which when stained with Congo red and viewed under polarizing microscope, give rise to characteristic green birefringence.

Sago spleen: Amyloid deposits in splenic follicles (white pulp).

Lardaceous spleen: Amyloid deposits in sinusoids of red pulp.

Liver

- **Gross:** It may cause moderate to marked enlargement. In advance stages, it appears pale, gray and waxy.
- **Microscopy**
 - Amyloid **first deposits in the space of Disse** and then progressively encroaches on adjacent hepatic parenchymal cells and sinusoids.
 - Progressive accumulation leads to deformity, pressure atrophy and disappearance of liver cells.

Heart

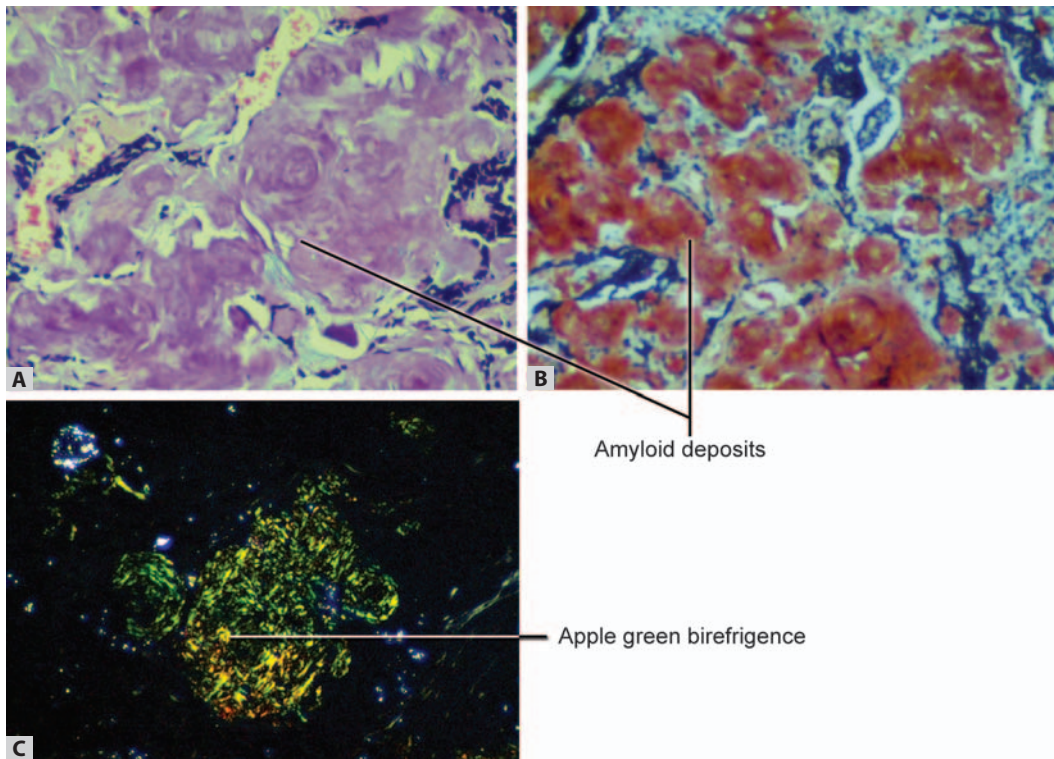
- It may be involved in **systemic amyloidosis (AL type)** or may be the major organ involved in **senile systemic amyloidosis**.
- **Gross:** Heart may be enlarged and firm. Subendocardial deposits may appear as gray-pink like dew-drop.
- **Microscopy**
 - **Myocardium:** Amyloid is deposited between the muscle fibers (Fig. 6.29) and their progressive accumulation causes pressure atrophy of myocardial fibers.

Other Organs

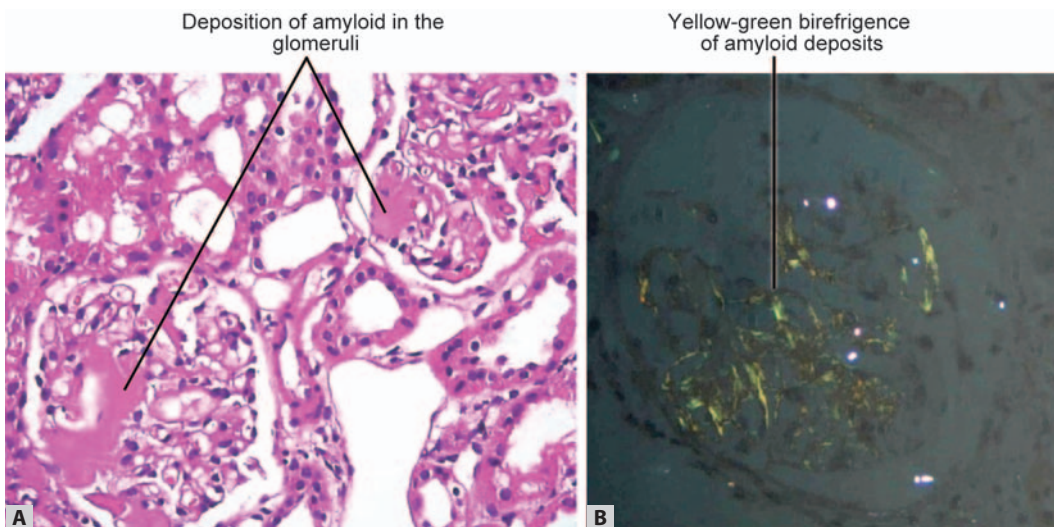
They may be involved in systemic disease and include adrenals, thyroid and pituitary. Nodular deposits in the tongue may cause **macroglossia**.



Fig. 6.26: Cut section of a amyloid kidney showing waxy appearance



Figs 6.27A to C: Amyloid deposits in medullary carcinoma of thyroid: (A) Amyloid appear as extracellular, amorphous, eosinophilic substance under H and E stain; (B) Congo red stain gives red color to the amyloid deposits; (C) Congo red stain viewed under polarizing microscope gives apple-green birefringence to amyloid deposits



Figs 6.28A and B: Amyloidosis of kidney: (A) Showing pink, amorphous extracellular amyloid deposits in the glomeruli; (B) Congo red stain showing apple-green birefringence under polarizing microscope

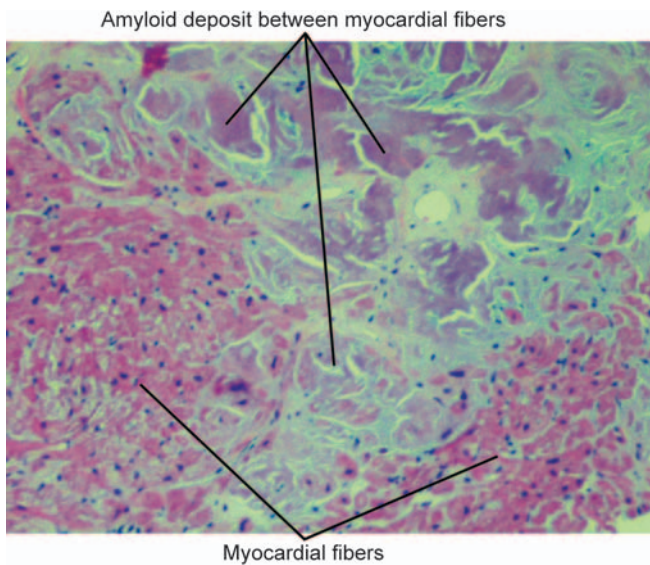


Fig. 6.29: Amyloid deposits between cardiac muscle fibers

Clinical Features

- Amyloidosis may not produce any clinical manifestations, or it may produce symptoms related to the sites or organs affected. Clinical manifestations **initially may be nonspecific** (e.g. weakness, weight loss). Specific symptoms appear later and are related to renal, cardiac and gastrointestinal involvement.
- **Renal involvement:** It gives rise to **proteinuria** sometimes massive enough to cause nephrotic syndrome. In advanced cases, the obliteration of glomeruli causes renal failure and uremia.

- **Cardiac amyloidosis:** It may present as **congestive heart failure**, conduction disturbances and arrhythmias.
- **Gastrointestinal amyloidosis:** It may be asymptomatic, or present as **malabsorption, diarrhea and digestive disturbances**. Amyloidosis of the tongue may hamper speech and swallowing.

Amyloidosis: Renal failure is a common cause of death with renal involvement.

Prognosis

- Generalized amyloidosis: Poor and poorer in myeloma-associated amyloidosis.
- Reactive systemic amyloidosis: Little better.

Diagnosis

Q. Write short note on diagnosis of amyloidosis.

It depends on the **histologic demonstration of amyloid deposits** in tissues.

- **Biopsy:** The most common sites are the **kidney, rectum or gingival tissues** in systemic amyloidosis.
- **Examination of abdominal fat aspirates** stained with Congo red is quite specific, but has low sensitivity.
- In immunocyte-associated amyloidosis, **serum and urine protein electrophoresis and immunoelectrophoresis** should be done. Bone marrow aspirates may show monoclonal plasmacytosis, even in the absence of multiple myeloma.
- **Scintigraphy with radiolabeled serum amyloid P (SAP)** component is a **rapid and specific test**.

Neoplasia

INTRODUCTION

Q. Define neoplasia.

Neoplasia literally means new growth, and a new growth formed is known as a neoplasm (Greek, *neo* = new + *plasma* = thing formed). The term “tumor” was originally used for the swelling caused by inflammation, but it is now used synonymously with neoplasm. Oncology (Greek, *oncos* = tumor) is the study of tumors or neoplasms.

Oncology: Study of neoplasms.

Willis definition: “A neoplasm is an **abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of the normal tissues and persists in the same excessive manner after cessation of the stimuli which evoked the change.**”

In the present era, a neoplasm can be defined as a disorder of cell growth which is triggered by a series of acquired mutations involving a single cell and its clonal progeny.

Salient Features of Neoplasia

- **Origin:** Neoplasms arise from cells that normally maintain a **proliferative capacity**.
- **Genetic disorder:** Cancer is due to **permanent genetic changes** in the cell, known as **mutations**. These mutations may occur in genes which regulate cell growth, apoptosis, or DNA repair.
- **Heritable:** The genetic alterations are passed down to the daughter tumor cells.
- **Monoclonal:** All the neoplastic cells within an individual tumor originate from a single cell/or clone of cells that has undergone genetic change. Thus, tumors are said to be monoclonal.

- **Carcinogenic stimulus:** The stimulus responsible for the uncontrolled cell proliferation **may not be identified or is not known**.
- **Autonomy:** In neoplasia, there is **excessive and unregulated proliferation** of cells that do not obey the normal regulatory control. The cell proliferation is **independent of physiologic growth stimuli**. But tumors are dependent on the host for their nutrition and blood supply.
- **Irreversible:** Neoplasm is irreversible and **persist even after the inciting stimulus is withdrawn or gone**.
- **Differentiation:** It refers to the **extent to which the tumor cells resemble the cell of origin**. A tumor may show varying degrees of differentiation ranging from relatively mature structures that mimic normal tissues (well-differentiated) to cells so primitive that the cell of origin cannot be identified (poorly differentiated).

Six Ps of neoplasm:

- Purposeless
- Progressive
- Proliferation unregulated
- Preys on host
- Persists even after withdrawal of stimulus (autonomous)
- Permanent genetic change in the cell.

Q. Discuss the nomenclature and classification of tumors.

CLASSIFICATION

Tumors are classified as **benign and malignant**, depending on the **biological behavior** of a tumor.

1. **Benign tumors:** They have relatively innocent microscopic and gross characteristics.
 - **Remain localized without invasion or metastasis.**
 - **Well-differentiated:** Their cells closely resemble their tissue of origin.

- **Prognosis:** It is **very good**, can be cured by surgical removal in most of the patients and the patient generally survives.
2. **Malignant tumors:** Cancer is the general term used for malignant tumors. The term “cancer” is derived from the Latin word for *crab*, because similar to a crab, malignant tumors adhere to any part that they seize on, in an obstinate manner.
- **Invasion:** Malignant tumors invade or infiltrate into the adjacent tissues or structures
 - **Metastasis:** Cancers spread to distant sites (metastasis), where the malignant cells reside, grow and again invade.
 - Exception: Basal cell carcinoma of the skin, which is histologically malignant (i.e. it invades aggressively), but rarely metastasize to distant sites. Glioma is malignant tumor of CNS.
 - **Prognosis:** Most malignant tumors cause death.

Malignant tumors:

1. Invasion/infiltration
2. Metastasis.

Almost all cancers can metastasize, except:

1. Basal cell carcinoma of skin
2. Glioma of CNS.

- **Importance of stroma:** It is required for growth, survival and replication of tumor (through blood supply) cells.
- **Tumor consistency depends on amount of stroma:**
 - **Soft and fleshy:** These tumors have scanty stroma.
 - **Desmoplasia** (Fig. 7.1 and refer Fig. 24.8): **Parenchymal tumor cells may stimulate the formation of an abundant collagenous stroma** → referred to as desmoplasia. For example, some carcinoma in female breast have **stony hard consistency** (or **scirrhous**).

Neoplasms: Consists of neoplastic parenchymal elements and non-neoplastic stroma.

Desmoplasia seen in:

- Some carcinomas (e.g. scirrhous) of female breast
- Cholangiocarcinoma
- Pancreatic cancer
- Linitis plastica (diffuse type of carcinoma of stomach).

NOMENCLATURE OF NEOPLASMS

Depending on the biological behavior, the tumors are classified as benign and malignant.

Microscopic Components of Neoplasms

Tumors (both benign and malignant) consist of two basic components:

1. **Parenchyma:** It is made up of neoplastic cells. The **nomenclature and biological behavior of tumors are based primarily on the parenchymal component of tumor.**
2. **Stroma:** It is the **supporting, non-neoplastic tissue derived from the host.**
 - **Components:** **Connective tissue, blood vessels and inflammatory cells** (e.g. macrophages and lymphocytes).
 - **Inflammatory reaction:** Stroma may show inflammatory reaction in and around the tumors. It may be due to ulceration and secondary infection in the tumors especially in the surface of the body. This type of inflammatory reaction may be acute, chronic or rarely granulomatous reaction. Some tumors show inflammatory reaction even in the absence of ulceration. It is due to cell-mediated immunologic response of the host against the tumor as an attempt to destroy the tumor. For example, lymphocytes in the stroma is seen in seminoma testis and medullary carcinoma of the breast.

Benign Tumors

They are generally **named by attaching the suffix “oma” to the cell of origin.**

Mesenchymal Tumors

They usually follow the below nomenclature (Table 7.1).

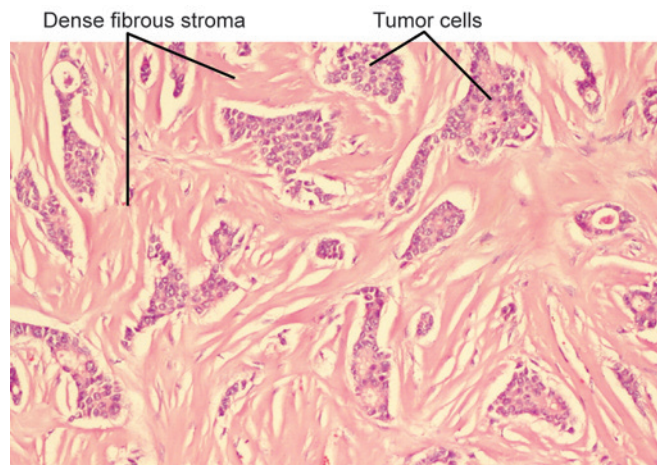


Fig. 7.1: Carcinoma of breast with abundant stroma separating malignant cells

Epithelial Tumors (Fig. 7.2)

Their nomenclature is **not uniform but more complex**. They are classified in different ways:

a. Cells of origin

b. Microscopic pattern

c. Macroscopic architecture.

- **Adenoma:** It is a **benign epithelial tumor arising from glandular epithelium**, although they may or may not form glandular structures.

Examples:

- ♦ **Adrenocortical adenoma:** It shows heterogeneous mass of adrenal cortical cells growing as a solid sheet without any glands. Termed adenoma because the cell of origin is glandular epithelium.
- ♦ **Follicular adenoma of thyroid:** It usually shows microscopically numerous tightly packed small glands (Figs 7.2A and 25.10).
- ♦ **Adenomatous polyp of the colon:** They are named because of gross appearance as a polypoidal lesion, which projects above a surface (refer Fig. 18.32).
- **Papilloma:** It is a **benign epithelial neoplasm** that produces microscopically or macroscopically **visible finger-like, exophytic or warty projections** from epithelial surfaces. Example: squamous papilloma (Fig. 7.2B).

- **Cystadenoma:** It is a tumor forming large cystic masses. Example: Serous cystadenoma of ovary (Fig. 7.2C).
- **Papillary cystadenoma:** It is a tumor which consists of papillary structures that project into cystic spaces. Example: Papillary serous cystadenoma of ovary (Figs 7.2D and refer 23.19 and 23.21).

Polyp (Fig. 7.3): It is a neoplasm that grossly produces **visible projection above a mucosal surface and projects into the lumen**. It may be either **benign or malignant**. It may have a stalk (**pedunculated polyp**) or may be without a stalk (**sessile polyp**). Example: Polyp of stomach or intestine.

Benign tumors:

1. Resemble the tissue of origin—**well-differentiated**
2. **Slow growing**
3. **Capsulated or well-circumscribed**
4. **Localized** to the site of origin.

Adenoma: Benign epithelial tumor arising from glands or forming glandular structures.

Papilloma: Benign tumor with visible finger-like projections.

Polyp: Tumor produces visible projection above mucosal surface and protrudes into the lumen.

Polyp: May be benign or malignant.

TABLE 7.1: Nomenclature of few benign and malignant mesenchymal tumors

Cell of origin	Benign	Malignant
Fibrous tissue	Fibroma	Fibrosarcoma
Fat cell	Lipoma	Liposarcoma
Blood vessel	Hemangioma	Angiosarcoma
Cartilage	Chondroma	Chondrosarcoma
Bone	Osteoma	Osteogenic sarcoma
Smooth muscle	Leiomyoma	Leiomyosarcoma
Skeletal muscle	Rhabdomyoma	Rhabdomyosarcoma

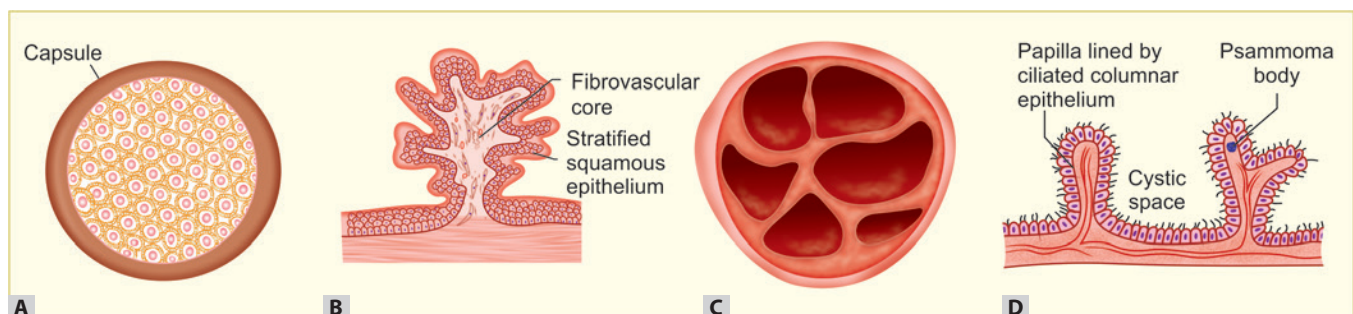
Malignant Tumors

They are termed as carcinoma or sarcoma depending on the parenchymal cell of origin.

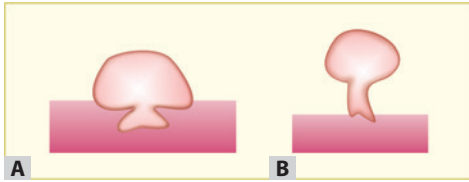
Q. Write short note on differences between carcinoma and sarcoma.

Sarcomas

They are **malignant tumors arising in mesenchymal tissue**. These tumors have little connective tissue stroma and are



Figs 7.2A to D: Morphological (gross/microscopic) appearance of some benign epithelial tumors. (A) Microscopy of adenoma composed of uniform glands (e.g. follicular adenoma of thyroid); (B) Squamous papilloma composed of finger-like projections; (C) Cut section showing cystadenoma (e.g. mucinous cystadenoma of ovary); (D) Microscopy of papillary cystadenoma composed of papillae projecting into the cystic cavity.



Figs 7.3A and B: Gross types of polyps: (A) Sessile polyp without a stalk; (B) Pedunculated poly with stalk

fleshy (Greek, *sar* = **fleshy**). **Examples:** Fibrosarcoma, liposarcoma, osteosarcoma, chondrosarcoma, leiomyosarcoma and rhabdomyosarcoma.

Sarcoma: Malignant tumor derived from **mesenchymal tissue**.

Carcinomas

They are **malignant neoplasms arising from epithelial cell**, which may be derived from any of the three germ layers (Table 7.2).

- **Undifferentiated malignant tumor:** It is a malignant tumor composed of undifferentiated cells, where the cell of origin cannot be made out on light microscopic examination.
- **Carcinosarcoma:** It is a rare malignant tumor which shows mixtures of carcinomatous and sarcomatous elements.
- **Inappropriate terminology for malignant tumor** (Table 7.3): In certain malignant tumors, the terms suffix “oma” is inappropriately used and sounds like a benign tumor.

Carcinoma: Derived from squamous, transitional or glandular (adenocarcinoma) epithelium.

Tumors of the hematopoietic system are indicated by the suffix “emia”, e.g. leukemia—malignant proliferation of leukocytes.

Exceptions: Anemia is not a neoplasm.

Malignant tumors:

1. Well-differentiated to poorly differentiated
2. Grow faster
3. Poorly circumscribed
4. Invade the surrounding tissue
5. Metastasize to distant sites.

Eponymously Named Tumors

These tumors are named after the person who first described or recognized the tumor (Table 7.4).

TABLE 7.2: Nomenclature of carcinomas

Germ layer	Tissue/cell	Malignant tumor
Ectoderm	Epidermis	Squamous cell carcinoma
Mesoderm	Renal tubules	Adenocarcinoma
Endoderm	Lining of the gastrointestinal tract	Adenocarcinoma

TABLE 7.3: List of malignant tumors with suffix “oma”

Inappropriate terminology for malignant tumor	Site
Hepatoma	Liver
Melanoma	Skin
Seminoma/dysgerminoma	Testis/ovary
Lymphoma	Lymph nodes and extranodal lymphoid tissue
Mesothelioma	Pleura, peritoneum

TABLE 7.4: Examples of some eponymously named tumors

Eponymously named tumors	Cell or tissue of origin
Burkitt lymphoma	B-cell lymphoma
Ewing sarcoma	Neuroectodermal origin arises in the bone
Grawitz tumor	Renal cell carcinoma arising from renal tubular epithelium
Kaposi's sarcoma	Malignant neoplasm of vascular endothelium
Hodgkin lymphoma	Malignant tumor of post-germinal B-cells
Brenner tumor	Benign tumor arising from surface epithelium of ovary

Other Tumors

Mixed Tumors (Fig. 7.4)

They are **derived from a single germ layer** but show **divergent differentiation** along two lineages. Example: **Mixed tumor of salivary gland** (pleomorphic adenoma) is derived from a single clone (either myoepithelial or ductal reserve cell) and giving rise to two components, namely epithelial and myoepithelial (stromal elements) cells (refer page 471).

Teratomas

Q. Write short note on teratoma.

They are special types of mixed tumors **derived from totipotent germ cells** (normally present in **ovary, testis** and

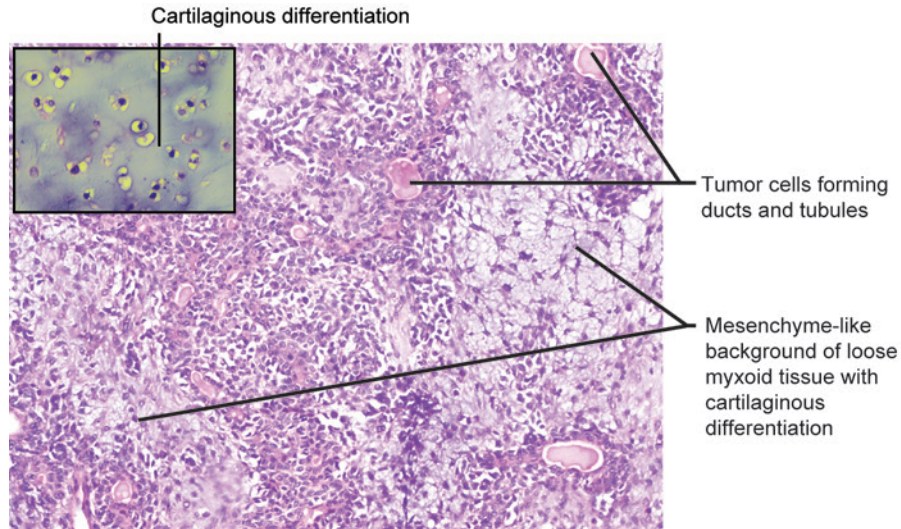


Fig. 7.4: Pleomorphic adenoma showing epithelial cells and myoepithelial cells separated by chondroid matrix. Inset shows cartilage

sometimes abnormally present in sequestered embryonic rest in midline). These cells have the capacity to **differentiate into any of the cell types** found in the adult body. Thus, teratoma contains recognizable **mature or immature cells** or tissues representative of more than one germ cell layer and sometimes all three. These cells or tissues are arranged in a helter-skelter fashion. The **tissue derivative from various germ cell layers** may include:

1. **Ectoderm** (e.g. skin, neural tissue, glia)
2. **Mesoderm** (e.g. smooth muscle, cartilage, bone, fat)
3. **Endoderm** (e.g. respiratory tract epithelium, gut, thyroid).

Teratoma: Derived from **totipotent cells** and contains tissues derived from ectoderm, endoderm and mesoderm.

Sites of teratoma:

1. Gonads
 - Ovary
 - Testis
2. Extragonadal, e.g. mediastinum.

Classification of Teratoma

- **Benign/mature teratoma:** It consists of **all mature and well-differentiated tissue**. Example: ovarian cystic teratoma (dermoid cyst), in which differentiation is mainly along ectodermal lines → produces a cystic tumor lined by skin with adnexal structure (hair, sebaceous glands) and tooth structures (refer Figs 22.11 and 23.25).
- **Immature/malignant teratoma:** It consists of **immature or less well-differentiated tissue**.
- **Monodermal teratoma and somatic-type tumors arising from dermoid cyst**, e.g. struma ovarii and carcinosarcoma developing in ovary.

- **Teratoma with malignant transformation:** It is the **development of malignant non-germ cell tumors** from one or more germ cell layer in a **teratoma**, e.g. squamous cell carcinoma developing in a teratoma of testis.

Hamartomas

Q. Write short note on hamartoma.

- It is a **disorganized mass of benign-appearing cells, indigenous to the particular site**.
- Example: Pulmonary chondroid hamartoma consists of islands of disorganized, but histologically normal cartilage, bronchi and vessels.

Hamartoma: Benign-appearing, non-neoplastic overgrowth of tissue.

Choristoma

Q. Write short note on choristoma.

- It is an **ectopic island of normal tissue—heterotopic rest** (normal tissue in an abnormal site) and is a **congenital anomaly**.
- Example: Presence of small nodular mass of normally organized pancreatic tissue in the submucosa of the stomach, duodenum, or small intestine.

Choristoma: Normal tissue in an abnormal site.

Embryonal Tumors (Blastomas)

They are the **type of tumor developed only in children** (usually below 5 years of age), and **microscopically resemble embryonic tissue** of the organ in which they arise (Table 7.5).

TABLE 7.5: Different types of embryonal tumors and their site

Type of embryonal tumor	Site
Retinoblastoma	Eye
Nephroblastoma or Wilms' tumor	Kidney
Neuroblastoma	Adrenal medulla or nerve ganglia
Hepatoblastoma	Liver

TABLE 7.6: Nomenclature of common tumors

Q. Write short note on histogenesis of tumors.

Tissue of origin	Benign	Malignant
Composed of single parenchymal cell type		
Tumors of Mesenchymal Origin		
Connective tissue and derivatives	Fibroma	Fibrosarcoma
	Lipoma	Liposarcoma
	Chondroma	Chondrosarcoma
	Osteoma	Osteogenic sarcoma
Vessels and surface coverings		
Blood vessels	Hemangioma	Angiosarcoma
Brain coverings	Meningioma	Invasive meningioma
Nerve sheath	Neurofibroma, neurilemmoma	Malignant peripheral nerve sheath tumor
Blood Cells and Related Cells		
Hematopoietic cells		Leukemia
Lymphoid tissue		Lymphoma
Muscle		
• Smooth muscle	Leiomyoma	Leiomyosarcoma
• Striated muscle	Rhabdomyoma	Rhabdomyosarcoma
Tumors of Epithelial Origin		
Stratified squamous	Squamous cell papilloma	Squamous cell carcinoma
Basal cells of skin or adnexa		Basal cell carcinoma
Epithelial lining of glands or ducts or organs	Adenoma	Adenocarcinoma
	Papilloma	Papillary carcinoma
	Cystadenoma	Cystadenocarcinoma
	Papillary cystadenoma	Papillary cystadenocarcinoma
Urinary tract epithelium (transitional)	Transitional-cell papilloma	Transitional-cell carcinoma
Tumors of melanocyte	Nevus	Malignant melanoma
More than one neoplastic cell type—mixed tumors, derived from one germ cell layer		
Salivary glands	Pleomorphic adenoma (mixed tumor) of salivary origin	Malignant mixed tumor of salivary gland origin
More than one neoplastic cell type derived from more than one germ cell layer		
Totipotent cells in gonads or in embryonic rests	Mature teratoma, dermoid cyst	Immature teratoma

Nomenclature of the more common forms of neoplasia is listed in Table 7.6.

Most of the malignant tumors kill, whereas benign tumors are usually not fatal.

CHARACTERISTICS OF BENIGN AND MALIGNANT NEOPLASMS

Q. Describe the characteristics of malignant tumors.

It is **very important to differentiate** benign from malignant tumors mainly because of the different prognostic outcome. In general, benign and malignant tumors can be distinguished on the basis of four fundamental features, namely: (1) Differentiation and anaplasia, (2) rate of growth, (3) local invasion, and (4) metastasis.

Differentiation and Anaplasia

Differentiation

Defined as the **extent to which neoplastic parenchymal cells resemble** the corresponding **normal parenchymal cells**. This includes both morphological and functional differentiation. Differentiation **determines the grade of the tumor**.

Benign Tumors

- **Well-differentiated:** The neoplastic cell **closely resembles the normal cell of origin**. It may be not possible to recognize it as a tumor by microscopic examination of individual cells (e.g. lipoma). Only the growth of these cells into discrete lobules discloses the neoplastic nature of the lesion (Fig. 7.5).
- **Mitoses:** They are **rare** and of normal configuration.

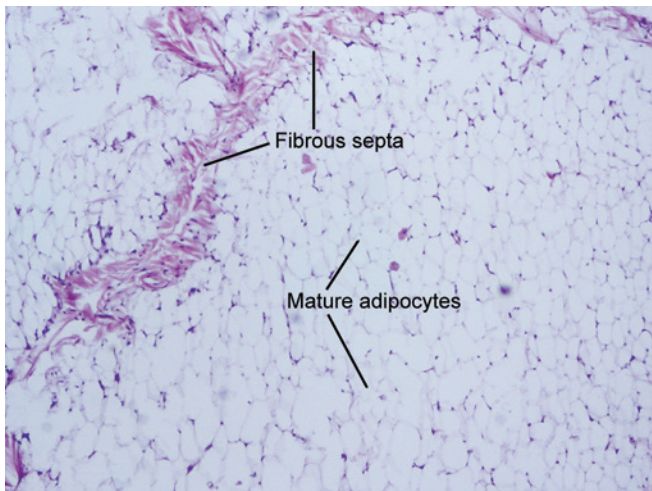


Fig. 7.5: Lipoma is a benign, well-differentiated tumor composed of lobules of fat cells that are identical in appearance to normal fat cells

Malignant Neoplasms

- Show a **wide range of differentiation** of parenchymal cells.
- Varies from **well-differentiated to completely undifferentiated**.
- Cancers are usually graded either as well, moderately or poorly differentiated or numerically often by strict criteria as grade 1, grade 2 or grade 3.
- **Well-differentiated tumors:**
 - Well-differentiated adenocarcinomas of colon may form normal-appearing glands (Fig. 7.6).
 - Squamous cell carcinomas may show cells which appear similar to normal squamous epithelial cells (Fig. 7.7).
- **Poorly differentiated tumors:** They consist of cells that have little resemblance to the cell of origin.
- **Moderately differentiated:** These tumors show differentiation in between the well and poorly differentiated tumors.

Anaplasia

Q. Write short note on anaplasia.

- Anaplasia **literally means “to form backward/backward formation”, i.e. reversal of differentiation of cell to a more primitive level**.
- Malignant neoplasms composed of undifferentiated cells are called as anaplastic tumors.
- **Lack of differentiation** (both structural and functional) is called as **anaplasia** and is **characteristic of malignancy**.
- The degree of anaplasia in a cancer cell correlates with the aggressiveness of the tumor.
- Thus, more anaplastic the tumor, the more aggressive it becomes.

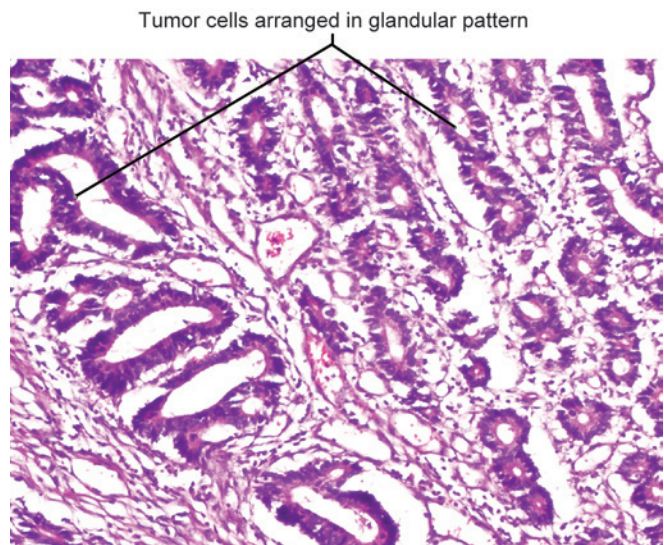


Fig. 7.6: Well-differentiated adenocarcinoma of the colon. It shows cancerous glands that are irregular in shape and size invading the muscular layer of the colon

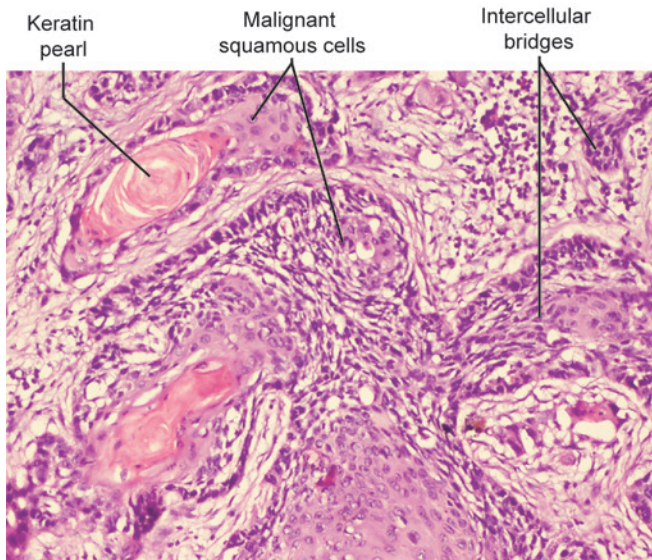


Fig. 7.7: Well-differentiated squamous cell carcinoma of the skin. The tumor consists of cells which are similar to normal squamous epithelial cells, with intercellular bridges and keratin pearls

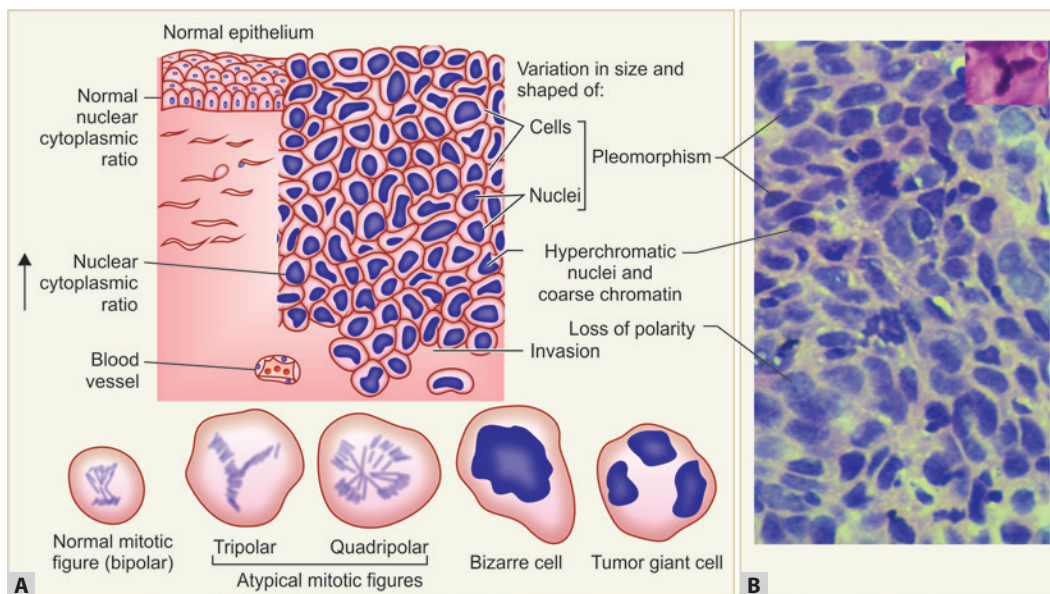
Microscopic features of anaplasia (Fig. 7.8):

Q. Write short note on morphology of malignant cells.

- **Pleomorphism:** It is defined as **variation in the size and shape of cells and cell nuclei**. It is a feature of malignancy. Thus, cells within the same malignant tumor range from large cells (many times larger than the neighbor cells), to extremely small (primitive appearing) cells.

• Abnormal nuclear morphology:

- **Extremely hyperchromatic nuclei** of tumor cells are due to **abundant chromatin** and **increased amount of DNA per cell** compared to that of a normal cell. Microscopically these **nuclei stain darkly (hyperchromatic nuclei)**.
- **Nuclear shape and size is variable** and may be irregular. **Chromatin is coarsely clumped** and distributed along the nuclear membrane. **Large prominent nucleoli** are usually seen.
- **Mitoses:** Presence of mitotic figures **indicates the higher proliferative activity** of the parenchymal cells.
 - ♦ **Number of mitotic figures:** Compared to benign and few well-differentiated malignant tumors, undifferentiated tumors usually show abundant (many) mitotic figures.
 - ♦ **Atypical (abnormal) mitotic figures** (Fig. 7.9): Normal mitosis produces bipolar spindles, and one cell divides into two. When the mitotic spindles are more than two, it is called as atypical. Presence of atypical bizarre mitotic figures is an important morphological feature of malignancy (See Fig. 7.8).
- **Nuclear cytoplasmic (N:C) ratio:** In a normal cell, N:C ratio is 1:4 or 1:6. In a malignant cell, the nuclei are enlarged, become disproportionately large for the cell, and the nuclear-to-cytoplasm ratio may be **increased** and may reach even up to 1:1.
- **Loss of polarity:** **Orientation of cells to one another** is known as polarity. The anaplastic cells lose the normal



Figs 7.8A and B: Microscopic features of anaplasia. A. Diagrammatic, B. Photomicrograph showing nuclear and cytoplasmic pleomorphism, hyperchromatic nuclei, high nuclear cytoplasmic ratio and loss of polarity. Inset of B shows tripolar mitotic figure

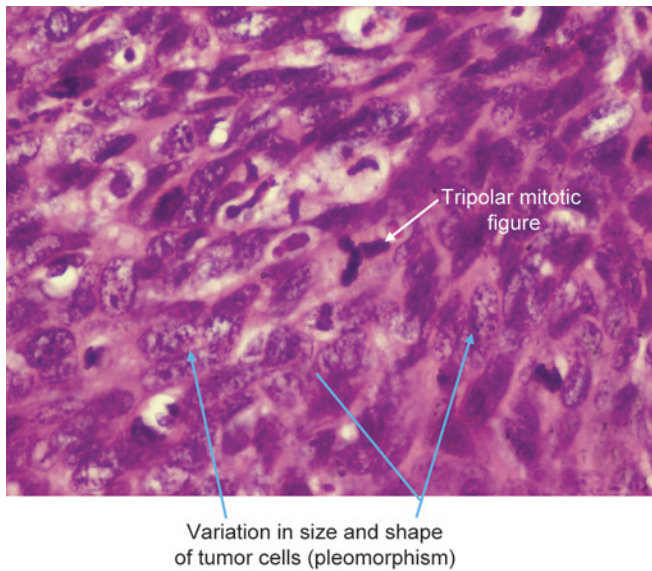


Fig. 7.9: Poorly differentiated carcinoma consisting of tumor cells showing variation in size of cells and nuclei. One tumor cell in the center shows an abnormal tripolar spindle

polarity → markedly disturbed orientation (architecture) of tumor cells.

- **Growth pattern:** Malignant neoplasms usually show disorganized growth. The tumor cells may form sheets of cells, arranged around blood vessels, papillary structures, whorls, rosettes, etc. Malignant tumors often show central ischemic necrosis due to compromised blood supply.
- **Bizarre cells, including tumor giant cells:** Some tumors may show **bizarre cells with a single large polymorphic nucleus** and others having **two or more large, hyperchromatic nuclei** (See Fig. 7.8).
- **Necrosis and apoptosis:** Many rapidly growing malignant tumors undergo large central areas of ischemic necrosis and/apoptosis.

Atypical mitotic figures: Produce tripolar, quadripolar, or multipolar spindles and these aberrant mitoses are incapable of complete cell division.

Functional Changes

Well-differentiated tumors usually retain the functional characteristics. Function may be in the form of secretion and vary depending on the tumor type.

1. **Secretion of normal substances:**
 - **Hormones:** Benign tumors and well-differentiated carcinomas of endocrine glands frequently secrete the hormones characteristic of their cell of origin (e.g. steroid hormones from an adrenocortical adenoma).

- **Normal product:** Example: **Well-differentiated squamous cell carcinomas** produce keratin → form characteristic **epithelial pearls**.
2. **Fetal proteins:** Some tumors may secrete fetal proteins, **which are not produced by comparable normal cells in the adult**. Example: Carcinoembryonic antigen (CEA) by adenocarcinomas of the gastrointestinal tract.
 3. **Ectopic hormones:** Tumors may produce **substances which are not indigenous to the tissue of origin** (refer pages 213–214). Example: Bronchogenic carcinomas may produce ACTH, parathyroid-like hormone, etc.

Anaplasia may be due to either backward differentiation or failure of differentiation.

Anaplasia: Some cancers arise from stem cells present in tissues. In these tumors, failure of differentiation rather than dedifferentiation (backward differentiation) is responsible for the undifferentiated appearance.

Neoplasms may secrete:

1. Normal hormones or products
2. Fetal proteins
3. Ectopic hormones.

Q. Write short note on differences between carcinoma and sarcoma.

Differences between carcinoma and sarcoma (Table 7.7).

Rates of Growth

Q. Write short note on rate of growth of tumors.

Factors Determining the Rate of Growth

1. **Degree of differentiation**
 - **Benign tumors** are well-differentiated and **usually grow slowly**.
 - **Most malignant tumors grow more rapidly**.
2. **Dependency:** Growth also depends on:
 - **Hormonal stimulation**, e.g. uterine leiomyomas may suddenly grow during pregnancy and may undergo atrophy after menopause.
 - **Adequacy of blood supply**.
3. **Balance between cell production and cell loss:** This in turn is determined by three main factors:
 - **Doubling time of tumor cells:** It is the **time required for the total cell cycle**, i.e. cell to double by mitosis.
 - **Growth fraction:** It is the **proportion of cells in the proliferative or replicative pool** within the tumor.
 - **Rate of tumor cell death:** Rate of growth depends on balance between cell production and cell loss. When

TABLE 7.7: Differences between carcinoma and sarcoma

Features	Carcinoma	Sarcoma
Definition	Malignant tumor of epithelial origin	Malignant tumor of mesenchymal origin
Meaning of the term	"Carcinoma" came from the Greek word "karkinos" which means crab and "oma" which means growth	"Sarcoma" came from the Greek word "sarx" meaning flesh and "oma" which means growth
Site of origin	Mostly from inside lining of colon, breast and lung or prostate	Arise from musculoskeletal system, such as bones, muscle and connective tissues
Incidence	More common cancer (more than 90% of cancers)	Less common (less than 1%)
Age	More common in middle and old age	Can occur at any age
Rate of growth	Usually not very rapid	Usually rapid
Route of spread	Initially lymphatics and later hematogenous	Spread by satellite nodules Usually hematogenous and lymphatic spread is rare
Site of metastasis through blood	Liver, lung, brain, bone and adrenals	May spread to lungs
Gross appearance	Varies depends on the subtype (e.g. cauliflower-like in squamous cell carcinoma) carcinomas infiltrate all nearby structures (nerves, veins and muscles)	Fleshy, grow in ball-like masses and tend to push nearby structures such as arteries, nerves and veins away
Hemorrhage and necrosis	Usually not extensive	May be extensive
Microscopy	Pattern varies and parenchymal cells may be arranged in glands, acini, sheets, cords, papillae depending on the subtype	Tumor cells are arranged in different pattern depending on the subtype
Radio-sensitivity	High	Radio- resistance
Prognosis	Depends on the location and stage	Depends on location and stage
Examples	Carcinoma breast, squamous cell carcinoma of skin and mucus membranes, carcinoma stomach and colon	Osteosarcoma, chondrosarcoma, liposarcoma

both the rate of cell production and the rate of cell loss (by apoptosis) is high, it is termed as high cell turnover.

Latent period: Time period between the exposure of the cell to the carcinogenic agent (initiation) till the tumor becomes clinically detectable.

Purpose of debulking the tumor with surgery or radiation: To shift tumor cells from resting phase (G_0) into the cell cycle and these cells become susceptible to chemotherapy.

Local Invasion

Benign Tumors

- Localized:** Most benign tumors **grow as expansile masses** that remain localized to their site of origin.
 - No infiltration** into adjacent tissue or capsule (if present).
 - No metastasis.**
- Capsule** (Figs 7.10 and 7.11): It is a rim of compressed connective tissue derived mainly from the extracellular matrix of the surrounding normal tissue.

- Capsule** → **makes tumor palpable and movable mass** → can be surgically enucleated.
- Benign tumors without capsule (unencapsulated).**
Examples: Hemangiomas, uterine leiomyoma.

Malignant Tumors

Q. Write briefly on pagetoid spread and give example.

- Lack of capsule:** Malignant tumors are poorly demarcated from the surrounding normal tissue and lack true capsule.
- Invasion** (Figs 7.12 and 7.13): **Two most reliable features** that differentiate malignant from benign tumors are **local invasion and metastases.**

Local invasion:

- Invasion of adjacent tissue/organ:** The cancers may invade and destroy the adjacent tissues/organ.
- Tissues that resists invasion:** They include mature cartilage (e.g. epiphysis), elastic tissue of arteries.
- Pagetoid infiltration:** It is invasion within epithelium and is seen in Paget's disease of the nipple (refer Figs 24.12 and 24.13).

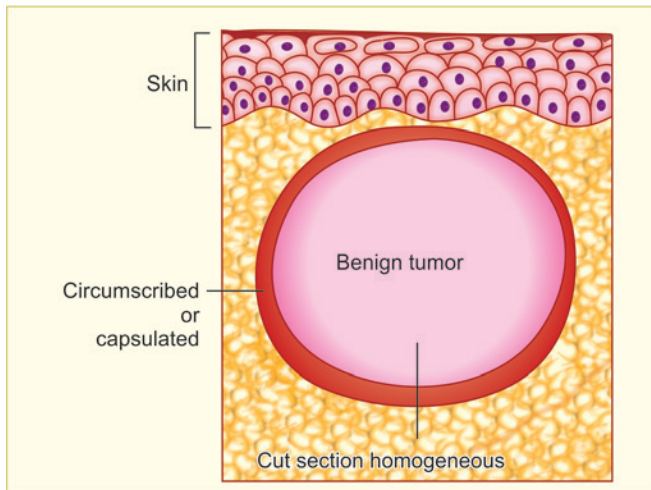


Fig. 7.10: Diagrammatic representation of a capsule in a benign tumor

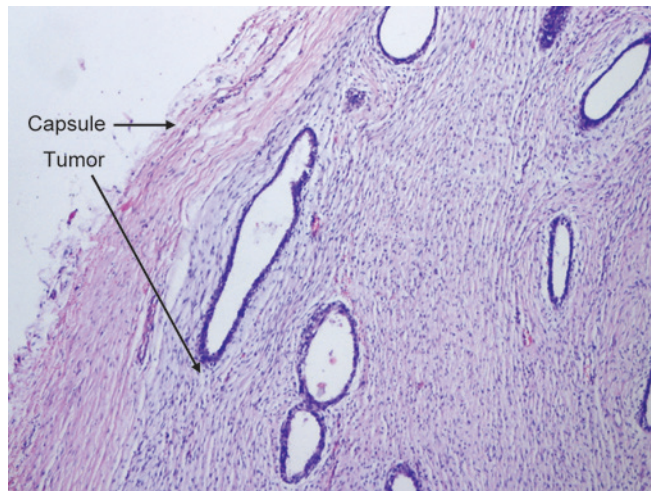


Fig. 7.11: Microscopic appearance of fibroadenoma of the breast with a well-defined fibrous capsule (left)

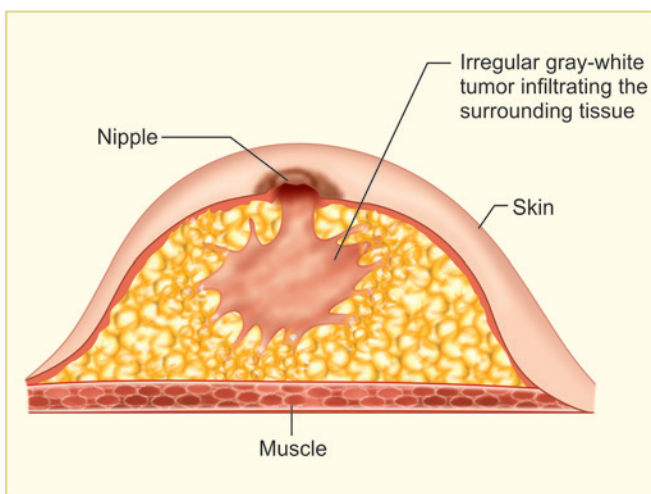


Fig. 7.12: Diagrammatic representation of cut section of an invasive ductal carcinoma of the breast

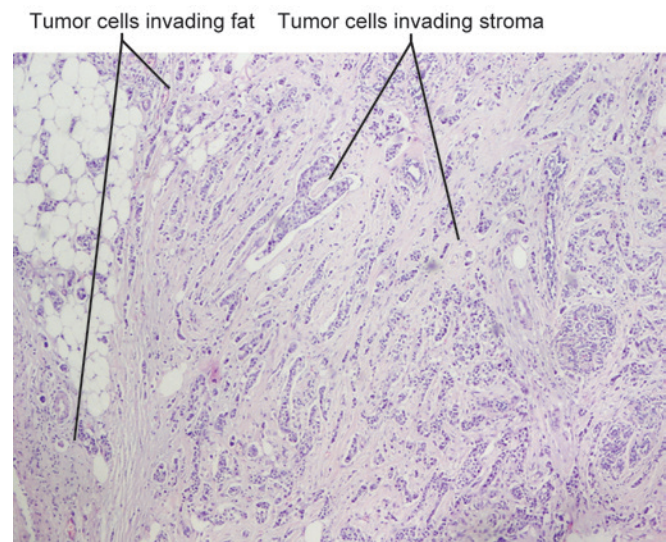


Fig. 7.13: Microscopic appearance of breast carcinoma showing invasion of breast stroma and fat by nests and cords of tumor cells

d. Invasion of blood vessels and lymphatics

e. **Perineural invasion:** For example, cancer of prostate and pancreas, adenoid cystic carcinoma of salivary glands.

Consequences of invasion into the organ/tissue of origin:

- Makes **surgical resection difficult**.
- **Functional insufficiency** may occur, if the much of normal tissue is replaced by cancer. Example: Hepatocellular carcinoma may cause liver insufficiency.
- **Compromise vital regions:** Brain tumors (e.g. astrocytomas, glioblastoma) may infiltrate and compromise vital regions.
- **Life-threatening location:** For example, intestinal obstruction due to carcinoma of colon.

Differences between benign and malignant tumors depends on:

- Differentiation
- Rate of growth
- Local invasion
- Metastasis.

Tissue relatively resistant to invasion: Cartilage and elastic tissue.

CARCINOMA IN SITU

Q. Write short note on carcinoma in situ.

Some carcinomas evolve from a **preinvasive stage** called as carcinoma in situ (refer Chapter 23).

Definition: Carcinoma in situ is defined as:

1. A **preinvasive epithelial neoplasm**.
2. Shows **all the cytological features of malignancy**.

3. **Involves the entire thickness of the epithelium.**
4. Remains **confined within the epithelial basement membrane.**

The tumor cells cannot reach the potential routes of metastasis, such as blood vessels and lymphatics until the basement membrane has been breached or invaded.

Carcinoma in situ: Lesion in which—

1. Dysplastic changes involve the entire thickness of the epithelium
2. Basement membrane is intact.

Dysplasia

Q. Write short note on dysplasia.

The cells that show cytological features of malignancy and the term dysplasia is used for these changes. It literally means disordered growth. The changes of dysplasia include:

1. **Cellular pleomorphism.**
2. **Large hyperchromatic nuclei.**
3. **High nuclear-to-cytoplasmic ratio.**
4. **Loss of polarity (architectural orientation).**

Classification of dysplasia: (1) **Mild**, (2) **moderate**, and (3) **severe** depending on the thickness of epithelium involved by the dysplastic cells.

Fate

- **Mild-to-moderate dysplastic changes**, which do not involve the entire thickness of epithelium **may be reversible**, if the cause is removed. Thus, **dysplasia need not progress to cancer.**
- **Once the tumor cells breach the basement membrane**, the tumor is said to be **invasive** and carcinoma in situ may take years to become invasive. Most in situ tumor, with time penetrate the basement membrane and invade the subepithelial stroma.

Sites: Uterine cervix, skin and breast.

Asymptomatic: In this stage, tumors are usually asymptomatic.

Metaplasia: It is reversible change in which one type of differentiated cell is replaced by another type of differentiated cells (refer pages 9 to 10). It is a cellular adaptation that develops in association with tissue damage, repair and regeneration. Examples: Gastroesophageal reflux damages the squamous epithelium of the esophagus which is replaced by glandular (gastric or intestinal) epithelium, columnar epithelium of endocervix is replaced by stratified squamous epithelium. Malignancy may develop in these metaplastic epithelium.

Dysplasia: Potentially reversible condition having intact basement membrane.

METASTASIS

Q. Define metastasis.

Q. Write short essay/note on mode of spread of malignant tumors /Discuss the different modes of metastasis with examples.

Definition: Metastases are tumor deposits discontinuous with the primary tumor and located in a distant tissue. This process is known as metastasis and the resulting secondary deposits are called metastases.

Metastasis is the process and the resulting secondary deposits are called metastases.

Significance

1. Metastases clearly **identify a tumor as malignant** because benign neoplasms never metastasize. **Exceptions** include two malignant tumors, which are locally invasive, but rarely metastasize.
 - **Gliomas** (malignant neoplasms of the glial cells) in the central nervous system.
 - **Basal cell carcinomas** of the skin.
2. Metastases **strongly reduce the possibility of cure of cancer.**
3. Metastatic spread is the most **common cause of cancer death.**

Factors favoring metastasis: (1) Poorly differentiated tumor, (2) more rapidly growing tumor, and (3) large primary tumor.

Metastases: First important criteria for malignancy.

Morphological Appearance

- Microscopically, **metastases resemble the primary tumor.** But occasionally, they may be so anaplastic that their cell of origin cannot be made out.
- **Unknown primary:** Sometimes metastases may appear without any clinically detectable primary tumor and the even microscopic examination of metastases may not reveal the characteristic features of primary site tumor. Example: Metastases from adenocarcinoma may be so anaplastic that there is no evidence of any gland formation. In such situations, **electron microscopic examination, immunohistochemistry** by specific tumor markers will be **helpful to establish the primary tumor.**

Pathways of Spread

Pathways of metastases: Lymphatics, hematogenous, spread along body cavities, direct transplantation, and rarely along epithelial lining.

Invasiveness of cancers allows them to penetrate blood vessels, lymphatics and body cavities. It provides an opportunity for spread/dissemination of cancers through the following pathways:

Lymphatic Spread

Q. Write short note on lymphatic spread of malignant tumors.

- **Most common pathway** of spread for carcinomas.
- **Regional node involvement:** The walls of lymphatics in the region of cancer are readily invaded by cancer cells and form a continuous growth within the lymphatic channels (lymphatic permeation). Once the tumor cells gain access into the lymphatic vessels, they may detach to form tumor emboli and are carried to the regional draining lymph nodes. In the lymph node, the **tumor emboli** enter through afferent lymphatics at its convex surface and lodge and grow in the subcapsular sinus. Subsequently, the entire lymph node may be replaced by the metastatic tumor.
- **Pattern of lymph node involvement follows the natural routes of lymphatic drainage.**
- **Sentinel lymph node** biopsy is done to know the presence or absence of metastatic lesions.

Q. Write short note on skip and retrograde metastasis.

- **Skip metastasis:** When **local lymph nodes are bypassed** and **lymphatic metastases develop in lymph nodes distant from the site of the primary tumor**; these are called "skip metastasis". Example: **Abdominal cancers** may be **first detected by an enlarged supraclavicular node**. **Virchow's lymph node** is metastasis to supraclavicular lymph node from cancers of abdominal organs (e.g. cancer stomach).
- **Retrograde metastasis:** **Tumors spreading against the flow of lymphatics** may cause metastases at unusual sites. Example: Carcinoma prostate metastasizing to supraclavicular lymph node.
- **Microscopic pattern of deposits:**
 - Initially, **tumor cells** are deposited in the **marginal sinus** and later extend throughout the node.
 - **Micrometastases** (microscopic involvement of lymph nodes) consist of **single tumor cells or very small clusters**.
- **Significance of lymph node metastases:** **Prognostic value**, e.g. in breast cancer, involvement of axillary

lymph nodes is very important for assessing prognosis and for type of therapy. However, all regional nodal enlargements need not be due to metastasis because necrotic products of tumor and antigens may produce sinus histiocytosis.

A historical emphasis on lymphatic spread for carcinomas and hematogenous spread for sarcomas may not always be true and both can spread by any route.

Lymph nodes: First line of defense in malignant tumors and most common site for metastases.

Sentinel lymph node is the first node in a regional lymphatic drainage that receives lymph flow from the primary tumor.

Hematogenous Spread

Q. Write short note on hematogenous spread of malignant tumors.

Hematogenous spread is usual for sarcomas but is also found in carcinomas. Blood borne metastasis usually occurs in **osteosarcoma, choriocarcinoma** and **renal cell carcinoma**.

- **Vessels invaded:** Cancer cells easily invade **capillaries and venules**, but thick-walled arterioles and arteries are relatively resistant.
- **Tumors with affinity for venous invasion:**
 - **Renal cell carcinoma:** It can invade the renal vein and grow in a snake-like fashion up the inferior vena cava, sometimes reaching the right side of the heart.
 - **Hepatocellular carcinoma:** It may invade branches of portal and hepatic vein and grow within the main venous channels.
- **Pattern of involvement:** With venous invasion, the pattern of metastases follow the venous flow.
- **Target organ for metastasis:**
 - **Liver and lungs:** They are the **most frequently involved** organs; liver, because **all portal area drains to the liver**. Tumors which penetrate systemic veins, eventually drain into the vena cava. Since **all caval blood flows to the lungs**, it is the other common site for secondaries by hematogenous spread.
 - Through pulmonary veins, cancer cells from the primary lung cancer and metastatic deposit in the lungs may be carried to the left side of the heart. From here the tumor emboli may be carried in systemic circulation to form secondary masses elsewhere in the body.
 - **Bone metastasis:** Cancer metastasizing to bone-prostate, lung, breast, liver, intestine, kidney and thyroid.

- ♦ Vertebral column is the common site and spread through the paravertebral plexus. Example: Carcinomas of the thyroid and prostate.
- ♦ Radiograph appearance of bone metastasis
 - ◊ **Osteolytic lesion:** It is characterized by radiolucencies (e.g. lung cancer) and may lead to pathological fractures and hypercalcemia.
 - ◊ **Osteoblastic lesion:** It is characterized by radiodensities (e.g. prostatic cancer, breast, thyroid) and increased serum alkaline phosphatase due to reactive bone formation.
- **Other common sites:** **Brain** most common primary is lung cancer, kidney and adrenals.
- **Organs relatively resistant:** For example, skeletal muscle and spleen.

MORPHOLOGY

- **Gross appearance** (Fig. 7.14): Appear as multiple round nodules of varying sizes found throughout the organ.
- **Microscopy** (Fig. 7.15): The metastatic deposits generally resemble the structure of primary tumor.

Tumor with strong propensity for vascular invasion:

1. Renal cell carcinoma
2. Hepatocellular carcinoma.

Hematogenous metastasis to bone: Vertebra is the most common site involved through paravertebral venous plexes.

Bone metastasis: May be either osteoblastic (radiodense) or osteolytic (radiolucent).

Osteoblastic metastasis: Increased alkaline phosphate and is seen in **prostatic cancer**.

Osteolytic metastasis: → hypercalcemia → pathological fracture.

Seeding of Body Cavities and Surfaces

Q. Write short note on transcelomic spread.

1. Transcelomic spread:

- a. **Malignant tumor arising in organs adjacent to body cavities** (e.g. ovaries, gastrointestinal tract, and lung), **may seed body cavities**. The malignant cells may exfoliate or shed from the organ surfaces into the body cavities and cytological examination of this fluid may show malignant cells.
- b. Body cavities include peritoneal (most common), pleural cavities (common), pericardial (occasionally), joint space and subarachnoid space.
 - i. **Peritoneal cavity:** Example: (1) **Ovarian tumors**, such as primary carcinomas of surface epithelial origin and (2) **malignant GI tract tumors** may spread to involve peritoneal cavity → ascites.
 - ii. **Pleural cavity:** Peripherally situated **lung tumors** → pleural effusions.

Multiple secondary tumor deposits in the lung parenchyma

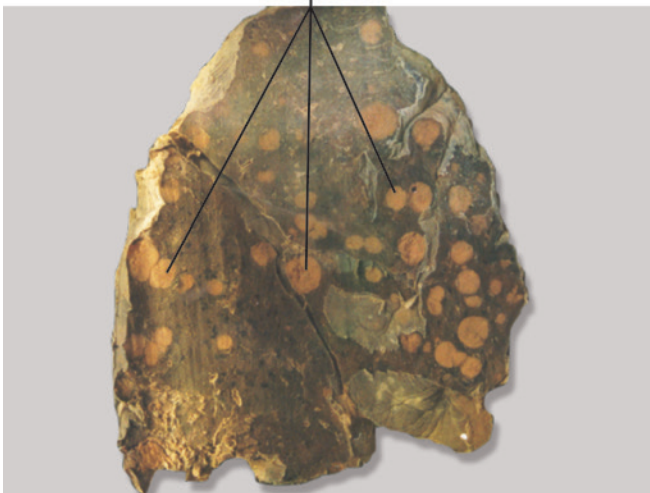


Fig. 7.14: Lung with multiple metastatic cancer

Liver tissue Malignant squamous cells deposited in the liver

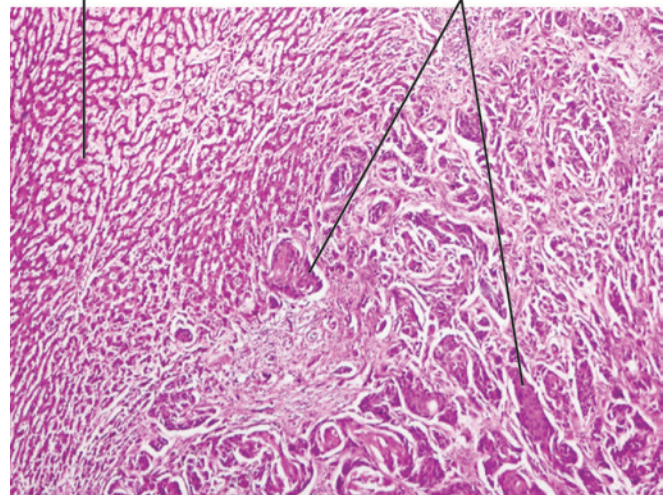


Fig. 7.15: Microscopic appearance of liver (left) metastasis from squamous cell carcinoma (right)

iii. **Cerebrospinal fluid: Glioblastoma** commonly spread through CSF in the subarachnoid space to the spinal cord.

2. **Spread along the epithelial lined spaces:** It is not common. Examples:
- Carcinoma endometrium may spread to ovary (or vice versa) through fallopian tube.
 - Carcinoma of kidney may spread to lower urinary tract via ureters.

Extranodal metastasis: Bad prognostic sign.

Drop metastasis: Medulloblastoma invades ventricles and spreads through CSF into spine.

Pseudomyxoma peritonei: Abundant mucin in the peritoneal cavity producing a gelatinous neoplastic mass occasionally seen in mucus-secreting appendiceal/ovarian carcinomas.

Direct Transplantation

- Tumor cells may be directly transplanted (e.g. by surgical instruments like scalpel, needles, sutures) or implantation by direct contact (e.g. transfer of cancer of lower lip to the corresponding opposite site in the upper lip).
- Even though this method is theoretically possible, they are **rare**.

Differences between benign and malignant tumors are summarized in Table 7.8.

TABLE 7.8: Differences between benign and malignant tumors

Q. Describe the differences between benign and malignant tumors.

Characteristics	Benign	Malignant
A. MICROSCOPIC FEATURES		
1. Differentiation/anaplasia	Well-differentiated	Well to poorly differentiated. Anaplasia is characteristic
2. Pleomorphism	Usually not seen	Commonly present
3. Nuclear morphology	Usually normal	Usually hyperchromatic, irregular outline and pleomorphic
4. Nucleoli	Usually absent	Usual and prominent
5. Mitotic activity	Rare and if present they are normal bipolar	High and may be abnormal or atypical (tripolar, quadripolar, multipolar)
6. Tumor giant cells	Not seen	May be seen and show nuclear atypia
7. Nuclear cytoplasmic (N:C) ratio	Normal (1:4 to 1:6)	Increased (may be as much as 1:1)
8. Polarity	Maintained	Usually lost
9. Chromosomal abnormality	Not found	Usually seen
B. GROSS FEATURES		
1. Border/capsule	Mostly circumscribed or encapsulated	Usually poorly defined
2. Areas of necrosis and hemorrhage	Rare	Common, often found microscopically
C. CLINICAL FEATURES		
1. Rate of growth	Usually slow	Relatively rapid
2. Local invasion	Usually well-demarcated without invasion/infiltration of the surrounding normal tissues	Locally invasive, infiltrate surrounding normal tissue
3. Metastasis	Absent	Frequent
4. Biological behavior/prognosis	Usually prognosis is good	Prognosis is poor; usually death due to local invasion or metastatic complications

Sentinel lymph node: Useful for—

1. Breast cancer
2. Malignant melanoma
3. Cancer of vulva.

Exfoliation of malignant cells through serosa occurs in malignant surface tumors, e.g. ovarian cancer.

INVASION–METASTATIC CASCADE (MOLECULAR EVENTS IN INVASION AND METASTASIS)

Q. Discuss the mechanism of invasion and metastasis.

Q. Write short note on metastatic cascade.

Invasion and metastasis are characteristic of malignant tumors.

Definition: Invasion–metastatic cascade constitutes the entire sequence of events from the beginning of invasion to the development of metastasis.

Invasion: Second most important criteria for malignancy.

Phases

Invasion–metastatic cascade is a **complex multistep process**. It can be divided into two main phases, namely: (A) **invasion of the extracellular matrix (ECM)** and (B) **metastasis (vascular dissemination and homing of tumor cells)**.

Invasion of Extracellular Matrix (Fig. 7.16)

Tumor cells must interact with ECM (includes basement membrane and interstitial tissue) at several steps in the

invasion–metastatic cascade. Invasion of the ECM is an active process and consists of four steps:

1. **Loosening of tumor cells:** Normal cells are attached to each other by adhesion molecules namely E-cadherins.
 - **Reduced/loss of E-cadherin function:** It is observed in most epithelial cancer (e.g. adenocarcinomas of the colon and breast) → **loosening of tumor cells**. The separated cells get detached from the primary cancer.
2. **Local degradation/proteolysis of basement membrane and interstitial connective tissue:** Extracellular matrix is of two types, namely: (1) Basement membrane and (2) interstitial connective tissue.
 - **Secretion of degrading enzymes:** Malignant tumor cells and stromal cells (e.g. fibroblasts and inflammatory cells) in the cancers secrete/induce many proteolytic enzymes that degrade ECM. These enzymes includes: **Matrix metalloproteinases (MMPs)**, **cathepsin** and **urokinase plasminogen activator (u-PA)**.
 - **Local degradation of basement membrane and interstitial connective tissue:** This is achieved by proteolytic enzymes.
3. **Changes in attachment/adhesion of tumor cells to ECM proteins:** Normal epithelial cells have receptors (e.g. integrin) for basement membrane components

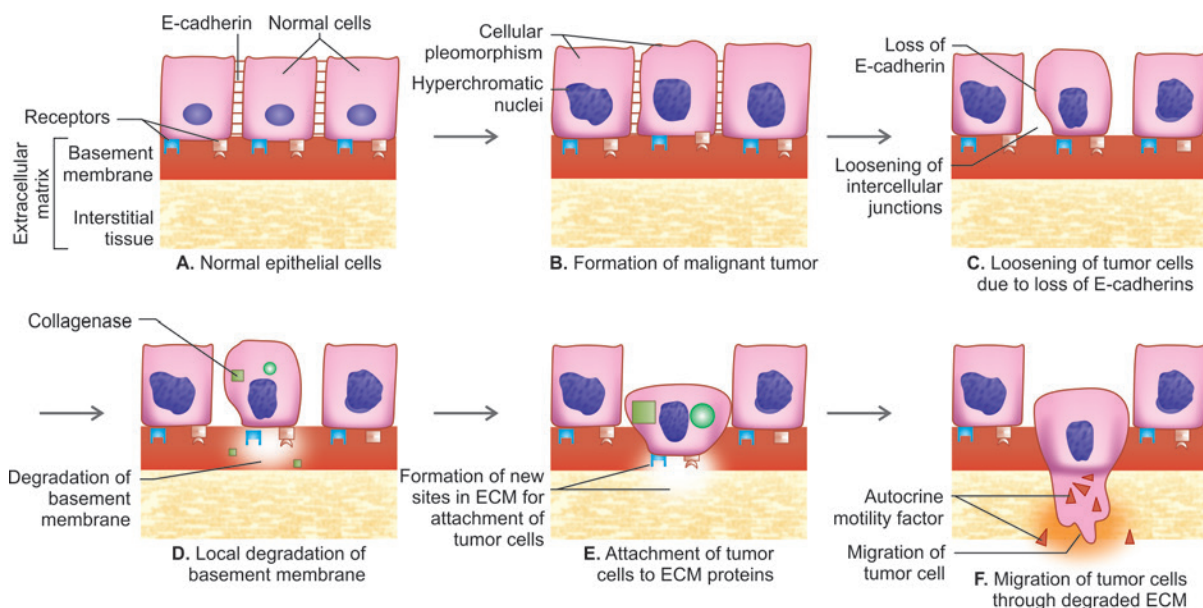


Fig. 7.16: Various steps in the invasion of extracellular matrix in invasion–metastasis cascades: (A) Normal cells; (B. to F) Tumor cells loosen and detach from each other because of reduced adhesiveness. The tumor cells bind components of the extracellular matrix and secrete proteolytic enzymes that degrade the extracellular matrix. With binding to proteolytically generated new binding sites in the ECM, tumor cell migration follows. The tumor cells reach the nearby vessels to start the next phase, namely metastasis

(e.g. laminin and collagen) and are located at their basal surface.

- **Generation of new sites:** Local degradation of basement membrane generates **new and strange sites** in the basement membrane.
 - **Adhesion of tumor cells to ECM:** The receptors on tumor cells attach to the new sites in the basement membrane.
 - **Stimulation of tumor cell migration:** It follows attachment/adhesion of tumor cells to ECM proteins.
4. **Locomotion/migration of tumor cells through degraded ECM:** It is a multistep process.
- **Locomotion/migration** drives the tumor cells **forward through the degraded basement membranes** and zones of proteolysis in the **interstitial connective tissue matrix**.
 - **Locomotion involves many receptors and signaling proteins.** The locomotion is potentiated by tumor cell-derived cytokines, such as **autocrine motility factors (AMF)** and other molecules.
 - **Migration through interstitial tissue:** The tumor cells invade and traverse through the surrounding interstitial connective tissue and ultimately **reach nearby blood and lymphatic vessels**. Cells gain access to the circulation by penetrating the basement membrane of vessels.

Loss of adhesive molecules→invasion.

Loss of E-cadherin: Leads to loosening of tumor cells.

Degradation of ECM: By proteolytic enzymes secreted by tumor cells and stromal cells.

Invasion steps:

1. Loosing of tumor cells
2. Local degradation of ECM
3. Attachment of ECM proteins
4. Migration of tumor cells.

Metastasis (Vascular Dissemination and Homing of Tumor Cells)

Metastasis is the process of deposition of tumor deposits away from primary.

Following the invasion of surrounding interstitial tissue, malignant cells may spread to distant sites by metastasis. Metastasis is **multistep process** by which tumor produces a secondary growth at a distant site or location. It has several steps (Fig. 7.17).

1. **Penetration of vascular or lymphatic channels (intravasation into the lumen of vessels):** Malignant cells penetrate the basement membrane of blood vessels or lymphatic channels.
2. **Invasion of the circulation and formation of tumor emboli:** In the circulation, tumor cells are susceptible to destruction by several of mechanisms. These include mechanical shear stress, apoptosis stimulated by loss of adhesion (termed *anoikis*), and innate and adaptive immune defenses. Survived tumor cells within the circulation, may clump with platelets to form platelet-tumor aggregates. This may enhance tumor cell survival and implantability. Tumor cells may also bind and activate coagulation factors and form emboli.
3. **Transit through the circulation.**

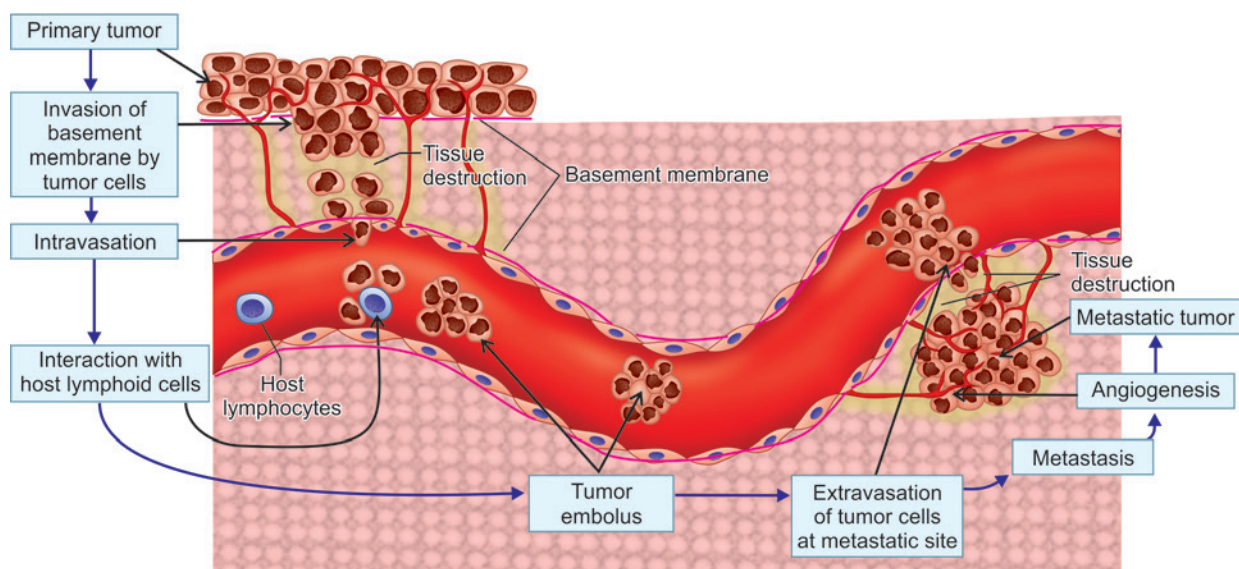


Fig. 7.17: Various steps involved in vascular dissemination and homing of tumor cells during the metastatic cascade

4. **Arrest within circulating blood or lymph:** It occurs at distant location away from primary tumor. At the site of arrest tumor cells adhere to endothelial cells.
5. **Exit from the circulation into a new tissue site:** Location at which circulating tumor cells leave the capillaries to form secondary deposits depends on the anatomic location and vascular drainage of the primary tumor and the tropism of particular tumors for specific tissues. Exit occurs through the basement membrane of lymphatics or blood vessel. The site at which circulating tumor cells leave the vessel or lymphatics must repeat the same events involved in invasion but in a reverse order.
6. **Formation of micrometastases:** Tumor cells lodge at a distant new site to form micrometastases. Examples for favored sites of metastasis:
 - Prostatic carcinoma to the bone.
 - Bronchogenic carcinomas to the adrenals and to the brain.
 - Neuroblastomas to the liver and bones.
7. **Angiogenesis.**
8. **Local growth of micrometastases into macroscopic tumor.**

Metastases: Some organs or tissues may be unfavorable soil for the growth of tumor deposit (e.g. spleen and skeletal muscle).

Various sites of metastasis and their most common sites of origin from primary tumor are listed in Table 7.9.

Two features that differentiate benign from malignant:

1. Local invasion
2. Metastases.

ENVIRONMENTAL FACTORS AND CANCER

Environmental factors are important **risk factors for most cancers**.

- **Smoking:** Cigarette smoking is an important factor involved in cancer of the lung, mouth, pharynx, larynx, esophagus, pancreas and bladder.
- **Alcohol abuse:** Alcohol abuse is a risk factor for carcinomas of the oropharynx (excluding lip), larynx and esophagus, and can produce alcoholic cirrhosis which is a risk factor for hepatocellular carcinoma. Alcohol and tobacco together increases the risk of cancers in the upper airways and digestive tract.
- **Infectious agents:** Example, *human papilloma virus* (HPV) spreads through sexual contact and is etiological factor for carcinoma of cervix as well as some head and neck cancers.
- **Obesity:** It is associated with cancer risk.

TABLE 7.9: Various sites of metastasis and their most common sites of origin from primary tumor

<i>Metastatic tumors in the organ</i>	<i>Most common site of primary</i>
Lung	From carcinoma of breast
Adrenal	From carcinoma of lung
Liver	From carcinoma lung > carcinoma colon > carcinoma pancreas > carcinoma breast > carcinoma stomach
Skin	In males: From carcinoma of lung In females: From carcinoma of breast Scalp is the most common site for cutaneous metastasis
Pancreas	From RCC > malignant melanoma On autopsy from carcinoma lung
Thyroid (rare)	Autopsy: From carcinoma of breast > Carcinoma of lung Pre-mortem: From RCC > Ca breast > Ca lung
Small bowel (metastatic tumors are more common than primary)	From: Other intra-abdominal organs From: Extra-abdominal source includes melanoma > carcinoma of breast > carcinoma of lung
CNS-Brain	From carcinoma of lung > carcinoma of breast
CNS-Leptomeninges	From carcinoma of breast
Esophagus	From carcinoma of lung
Spleen	From carcinoma of lung > carcinoma of breast > melanoma
Heart	Males: From carcinoma of lung Females: From carcinoma of breast
Testis	From carcinoma of prostate > carcinoma of lung > GI tract malignancies > melanoma > kidney
Penis	From carcinoma of bladder

- **Hormones:** Exposure to estrogen stimulation, if unopposed by progesterone, increases the risk of cancers of the breast and endometrium.
- **Carcinogens:** They may be present in food (e.g. grilled meat, high-fat diet, alcohol), water (e.g. arsenic), environment [e.g. ultraviolet (UV) rays, asbestos], drugs medications (e.g. methotrexate), etc.

Q. Write short note on diet and cancer.

- **Diet and cancer:** Though not proved, it may a risk factor for colorectal carcinoma, prostate carcinoma and breast carcinoma. **Three factors in the diet are probably involved in the development of cancer:**

- **Exogenous carcinogen in diet:** *Aflatoxin* causes a specific mutation in codon 249 of the *TP53* gene and is involved in the development of hepatocellular carcinomas. The role of food additives, artificial sweeteners, and contaminating pesticides in the genesis of cancer is not known.
- **Endogenous synthesis of carcinogens from dietary components:**
 - ♦ **Nitrosamines and nitrosamides:** It was implicated mainly in the genesis of gastric cancer. Nitrosamines and nitrosamides in the diet can induce gastric cancer. These compounds are formed in the stomach from nitrites and amines or amides from the digested proteins in the diet. Sources of nitrites include sodium nitrite (added as food preservative), and nitrates (present in common vegetables) and these are reduced to nitrosamine and nitrosamides in the gut by bacterial flora.
 - ♦ **High animal fat intake:** This along with consumption of red meat and low dietary fiber intake has been implicated in the causation of carcinoma colon. Probably high fat intake increases the bile acids level in the gut. This modifies intestinal flora and favors the growth of microaerophilic bacteria. Bile acid metabolites produced by the action of these bacteria may be carcinogenic.
- **Lack of protective factors**
 - ♦ **High-fiber diet** may have a protective role in carcinoma colon. This may be due to (1) increased bulk of stool and reduced transit time, which reduces the exposure of mucosa to probable carcinogens, and (2) certain fibers in the diet may bind to carcinogens and protect the mucosa. However, it is not proved.
 - ♦ Correlation between total **dietary fat intake** and breast cancer is also not clear.
 - ♦ **Antioxidant:** Fruits and vegetables, consumption of vitamin C and E, β -carotenes and selenium which have antioxidant properties and have been presumed to have anticarcinogenic effect. However, there is no convincing evidence that antioxidants act as chemopreventive agents. Retinoids are effective agents in the therapy of acute promyelocytic leukemia, and there are reports mentioning the associations between low levels of vitamin D and cancer of the colon, prostate and breast.
 - ♦ Epidemiologic studies suggest that a **folate-rich diet** decreases the risk of colorectal cancer.

In conclusion, dietary influences on cancer development are highly controversial. There is no definitive evidence to indicate that a particular diet can cause or prevent cancer. Association has been mentioned that physical activity decreases the risk of developing cancer of breast and colon whereas obesity increases the risk for endometrial, esophageal and kidney cancer.

PRECANCEROUS CONDITIONS/ PRECURSOR LESIONS

Q. Write short note on precancerous lesions/premalignant neoplasms.

Precancerous conditions (precursor lesions) are **non-neoplastic disorders** in which there is a **well-defined association with an increased risk of cancer**. However, in majority of these lesions no malignant neoplasm develops except that they have an increased risk. Examples:

1. **Chronic atrophic gastritis** of pernicious anemia.
2. **Solar or actinic keratosis of the skin, Bowen's disease of the skin.**
3. **Chronic inflammation:** Chronic ulcerative colitis (carcinoma colon), cirrhosis of liver (hepatocellular carcinoma), *H. pylori* gastritis (gastric cancer and lymphoma), chronic irritation from jagged tooth or ill-fitting denture (cancer of the oral cavity) and old burn scar—Marjolin's ulcer (squamous cell carcinoma).
4. **Leukoplakia** (erythroplakia) of the oral cavity, vulva and penis.
5. Barrett esophagus.
6. Squamous metaplasia and dysplasia of bronchial mucosa observed in chronic smokers. Intralobular and intraductal carcinoma of the breast, carcinoma in situ of cervix.
7. Endometrial hyperplasia and dysplasia in women with unopposed estrogen stimulation.
8. Precancerous benign tumors: Few forms of benign tumors may transform into malignant. Example: villous adenoma of the colon, as it increases in size, becomes malignant.
9. Benign develops occasionally into malignant: Most benign tumors do not become malignant. However, occasionally it may arise from benign tumors. Examples:
 - Leiomyosarcoma beginning in a leiomyoma.
 - Carcinoma developing in long-standing pleomorphic adenomas.

- Malignant peripheral nerve sheath tumor in patients with neurofibromatosis.

10. Congenital abnormalities may predispose to cancer. Example: The undescended testis is more prone to neoplasms than the normally located testis.
11. Immunodeficiency states: Patients with deficits in T-cell immunity have increased risk for cancers mainly those due to oncogenic viruses.

Increased risk of cancer is seen in:

- Chronic ulcerative colitis
- Chronic atrophic gastritis
- Solar keratosis
- Leukoplakia
- Barrett esophagus.

MOLECULAR BASIS OF CANCER

Fundamental Principles

1. **Cancer is a genetic disease** and arises through a series of somatic alterations in DNA that result in uncontrolled proliferation of cells with altered DNA.
2. **Nonlethal genetic damage** (mostly in DNA) **known as mutation is essential for carcinogenesis**, because lethal damage cause death of cells. Mutation may be:
 - **Inherited** in the germ line and occurs in certain families.
 - **Acquired** by the action of environmental agents (e.g. chemicals, viruses or radiation) and result in sporadic cancers.
3. **Tumors are monoclonal**, i.e. they originate from a clonal proliferation of a **single type of progenitor cell that has undergone genetic damage**.
4. **Carcinogenesis is a multistep process that occurs over time. This is the result of accumulation of complementary mutations.**
 - **Cancer hallmarks:** This represents **phenotypic properties of malignant neoplasms**. This includes excessive growth, local invasiveness and the ability to form distant metastases. These cancer hallmarks are due to genomic alterations which change the expression and function of key genes and thereby impart a malignant phenotype.
 - A relatively small number of genetic changes are fundamental to oncogenesis. **Mutations that produce malignant phenotype are referred to as “driver mutations”.** **Initiating mutation** is the **first driver mutation** that starts a cell on the path to malignancy.

It is typically maintained in all of the cells of the subsequent cancer. However, only one mutation usually do not fully transform the cell into cancer cell. Hence, development of a cancer requires that the “initiated” cell undergo a number of additional driver mutations, each of which also contributes to the development of the cancer.

- **Loss-of-function mutations in genes that maintain genomic integrity is a common early step in malignancy, particularly in solid tumors.** Mutations which lead to genomic instability increase the chances of acquiring driver mutations (that are needed for malignant behavior) and also greatly increase the frequency of mutations that have no phenotypic consequences (called “**passenger**” or “**hitchhikers**” mutations). Passenger mutations are much more common than driver mutations.
5. **Four classes of normal regulatory genes are the main targets of genetic damage.**
 - **Growth-promoting proto-oncogenes:** (Refer page 183).
 - **Growth-inhibiting tumor suppressor genes:** They normally **prevent uncontrolled growth** (Refer page 187).
 - **Genes involved in DNA repair:** (Refer page 197).
 - **Genes that regulate programmed cell death** (apoptosis): Refer page 193.
 6. **Failure to differentiate:** The cancer cells arrest at a stage before their terminal differentiation and may retain their stem cell properties.

Four types of genes involved in neoplasia:

1. Oncogenes
2. Tumor suppressor genes
3. DNA repair genes
4. Genes involved in apoptosis.

Loss-of-function mutation: Mutation that results in reduced or abolished protein function.

Gain-of-function mutations: Less common and causes abnormal activity of protein. It can take two forms:

1. Increase in a protein's normal function (e.g. excessive enzymatic activity) and
2. Impart a completely new activity unrelated to the affected protein's normal function.

GENETIC LESIONS IN CANCER

Genetic changes in cancer may be **minute** or **large** enough to be identified in a karyotype.

Karyotype Abnormalities in Tumors

These may be due to **abnormalities in: (a) structure or (b) number (aneuploidy)** in which whole chromosomes may be gained or lost.

Structural Abnormalities

Common structural abnormalities are: (1) balanced translocations, (2) deletions, (3) gene amplifications and (4) point mutations.

Mechanisms of mutations in tumor cells:

- Point mutations
- Balanced translocations
- Deletions
- Gene amplifications.

Balanced Translocations

- Associated with **hematopoietic** and **mesenchymal neoplasms**.
- **Method of activation of proto-oncogenes:** Balanced translocation can activate proto-oncogenes by two ways: (1) Overexpression or (2) forming chimeric gene.
 - **Overexpression** → loss of normal regulatory control on these genes. Example: In Burkitt lymphoma (Fig. 7.18), **most common translocation t(8;14)(q24;q32)** → converts MYC proto-oncogene into MYC oncogene → **overexpression of MYC protein** (oncoprotein) → **uncontrolled cell proliferation** and stimulation of apoptosis.

- **Forming chimeric gene** → chimeric proteins → cellular proliferation. For example, **Philadelphia (Ph) chromosome** in chronic myelogenous leukemia. **Balanced reciprocal translocation** between long arm of chromosomes 9 and 22, i.e. **t(9;22)(q34;q11.2)** → **shortened chromosome 22**—Philadelphia chromosome (refer Fig. 11.19). **ABL** (Abelson murine leukemia virus) proto-oncogene from chromosome 9 **joins** the **BCR** (breakpoint cluster region) on chromosome 22 → produces a new **chimeric (fusion) gene** → **called BCR-ABL oncogene** → **causes cell division and inhibition of apoptosis**.

Balanced translocation: Produces carcinogenesis either by overexpression of oncogenes or by forming chimeric gene.

Deletions

- Chromosomal deletions are more **common in non-hematopoietic solid tumors** and are the second most structural abnormality found in tumor cells.
- Deletion is **common with tumor suppressor gene** and causes **loss of particular tumor suppressor gene protein**. Example: Deletion of **RB** gene (involving chromosome 13q14) is associated with retinoblastoma.

Deletions: Frequently affect tumor suppressor gene.

Gene Amplification

Gene amplification: Increases the expression of oncogenes.

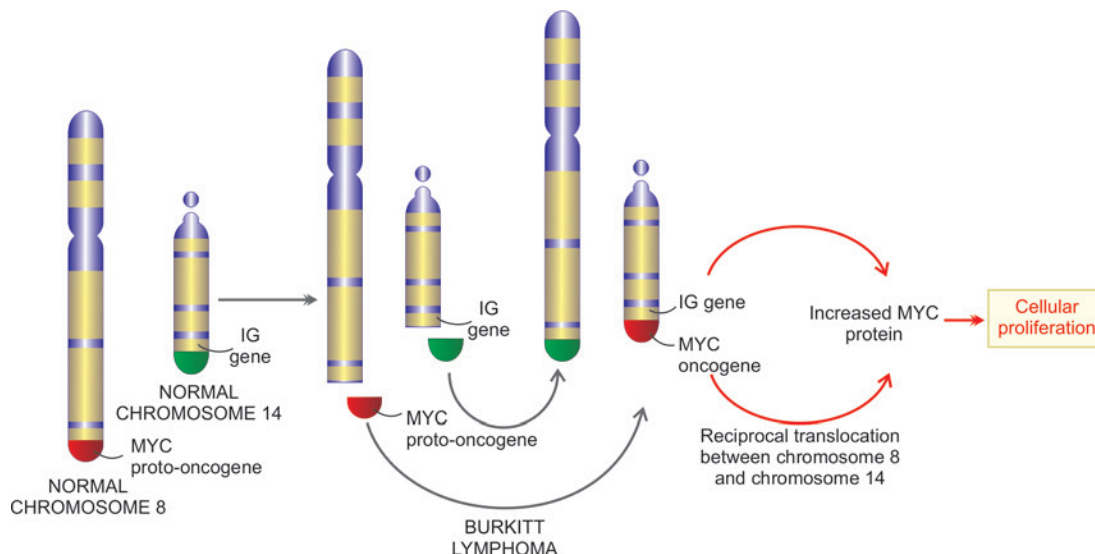


Fig. 7.18: Balanced translocation and activation of MYC oncogene in Burkitt lymphoma

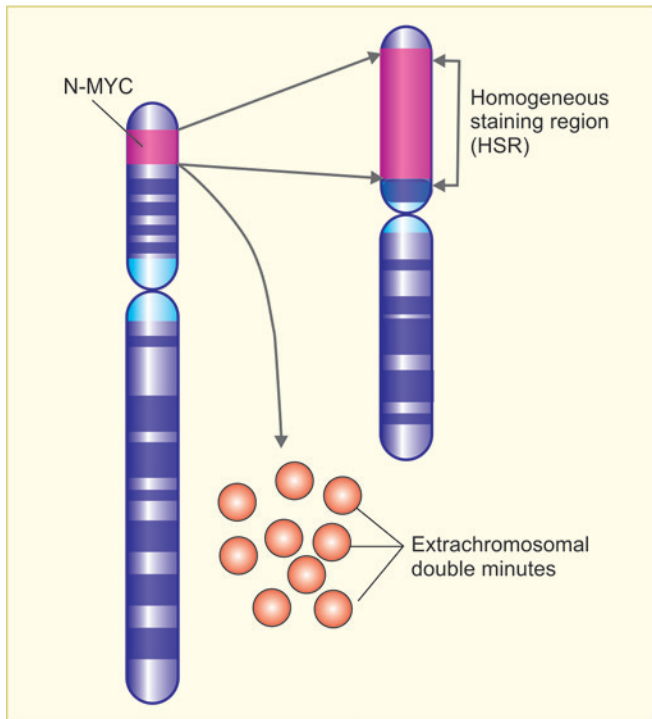


Fig. 7.19: Amplification of the N-MYC gene in human neuroblastomas seen either as extrachromosomal double minutes or as a chromosomally integrated, homogeneous staining region (HSR)

- Gene amplification is a chromosomal alteration in which there are an **increased number (several hundred copies) of gene copies**.
- Proto-oncogenes may be converted to oncogenes by gene amplification.
- Gene amplification → produces several hundred copies of the proto-oncogene in tumor cells → **overexpression of gene product (normal proteins)**.
- It has been found mainly in **human solid tumors**.
- Gene amplification can produce two patterns (Fig. 7.19):
 - **Extrachromosomal multiple, small, structures (called “double minutes”/dmins)**
 - Chromosome alterations referred to as **homogeneous stainable regions (HSR)** if increased copies of gene are integrated within chromosomes. Increased copies of gene may be inserted into new chromosomal location, which may be distant from the normal location of the involved genes. HSR appear as homogeneous in G-banded karyotype.
- Examples: (1) **N-MYC gene amplified in neuroblastomas** and associated with poor prognosis. (2) **HER2/Neu (also called ERBB2) amplification in breast cancer**.

Chromothripsis

Dramatic chromosome “catastrophes” are called chromothripsis (literally means chromosome shattering).

Chromothripsis is found in about 1–2% of cancers, up to 25% of osteosarcomas and other bone cancers and in gliomas. It probably develops as a single event in which **dozens to hundreds of chromosome breaks occur within part or across the entirety of a single chromosome or several chromosomes**. These catastrophic events may simultaneously activate oncogenes and inactivate tumor suppressors leading to carcinogenesis.

Numerical Abnormalities

Aneuploidy

Aneuploidy is the **presence of chromosome numbers that is not multiple of haploid number** (i.e. multiples of 23). It is common in cancers particularly in carcinomas.

Minute/Subtle Changes

- Genetic changes in cancer may be subtle and **cannot be detected by karyotyping**. These include: Point mutations or insertions and deletions.
- **Point mutation is characterized by substitution of a single nucleotide base by a different base in a gene** (refer page 218–219). It may change the code in a triplet of bases and lead to the replacement of one amino acid by another in the gene product. **Point mutation is a common mechanism of oncogene activation**. Examples: Point mutations in one of the *RAS* genes (*HRAS*, *KRAS* or *NRAS*) are observed in—85% of pancreatic cancers and 45% of colon cancer, point mutations of *RET* in leukemia and *BRAF* in melanoma.

Point mutations: Most common type of mutations seen in malignant tumors.

Epigenetic Modifications and Cancer

Epigenetic modifications in cancer may involve:

- Tumor suppressor genes
- DNA repair genes.

Definition: Epigenetics is a **reversible, heritable mechanisms that control gene expression independent of DNA base sequences and occurs without mutation**. It is unrelated to gene nucleotide sequence. **Epigenetics** is the mechanism that control gene expression.

- Epigenetic changes involve **histone modification** and **DNA methylation**, both of which affect gene expression.

- **In normal cells, the majority of the genome is not expressed, because they are silenced by DNA methylation and histone modifications.** Apart from DNA mutations, epigenetic aberrations are also responsible for the malignant properties of cancer cells.
- Epigenetic modifications are usually passed on to daughter cells and may occasionally result in changes in gene expression. **In cancer cells** there is global DNA hypomethylation and selective localized hypermethylation.
Examples:
 - **Silencing genes by hypermethylation:** (1) **Tumor suppressor gene *p53*, *BRCA1*** in breast cancer and ***VHL*** in renal cell carcinomas and (2) DNA repair genes: Mismatch-repair gene ***MLH1*** in colorectal cancer.
 - **Hypomethylation** → can cause chromosomal instability, derepression of growth regulatory genes, and overexpression of antiapoptotic genes → induce tumors.
 - **Changes in histones:** Cancer cells may show changes in histones near genes that influence cellular behavior.
 - Unlike DNA mutations, epigenetic changes are potentially reversible by drugs that inhibit DNA- or histone-modifying factors.

Noncoding RNAs and Cancer

It is observed that many genes do not encode proteins. Instead, their products play important regulatory functions.

One class of genes, which do not encode proteins but their products play important role in gene regulation, is small RNA molecules. They are small noncoding, single-stranded RNAs → called as microRNAs (miRs).

- **Role in carcinogenesis: Amplifications and deletions of miR loci** have been observed in many cancers. The miRs that promote tumor development are often called as ***onco-miRs***. miR-200 are important in invasiveness and metastasis; and miR-155, is overexpressed in many human B-cell lymphomas. Deletions affecting certain **tumor suppressive miRs**, such as miR-15 and miR-16, are frequent genetic lesions in chronic lymphocytic leukemia.
- **Mode of action:** (1) Increased expression of oncogenes or (2) reduced expression of tumor suppressor genes.

MicroRNAs (miRNAs): New classes of regulatory molecules which can act as either oncogenes or tumor suppressors. They affect the translation of other genes.

Deletion/loss of expression of miRNAs: Carcinogenesis by overexpression of proto-oncogenes.

Overexpression of miRNAs: Carcinogenesis by reducing expression of tumor suppressor genes

STEPS IN NORMAL CELL PROLIFERATION

Normal cell follows a controlled proliferation. The different sequential steps are:

1. **Growth factors binding** to its specific cell surface receptor.
2. **Transient and limited activation** of the growth factor receptor → activates signal-transducing proteins on the inner aspect of the cell membrane. Following this signaling, the receptor reverts to its resting state.
3. **Intracellular signal transduction:** Most of the signal-transducing proteins are located on the inner aspect of the plasma membrane. They **receive external signals** and get activated (by binding of growth factor to its growth factor receptors) and **transmit the growth signal across the cytoplasm → to the nucleus of the cell**. The most important signal-transducing protein belongs to **RAS family and ABL**.
4. **Transcription:** Activation of nuclear regulatory factors → initiates DNA transcription.
5. **Cell cycle:** Entry and progression of the cell into the cell cycle → resulting in cell division.

HALLMARKS OF CANCER

Normal cell may undergo malignant transformation by corrupting any one of the normal steps involved in cell proliferation.

1. Increased action of positive growth regulators.
2. Loss of function of negative growth regulators.
3. Altered cellular metabolism
4. Loss of normal apoptosis pathways.
5. Loss of replicative senescence.
6. Increased angiogenesis.
7. Ability to invade and metastasize (refer page 190).
8. Evasion of the host immune response.

Deregulated cell proliferation: Increased action of positive growth regulators (oncogenes, i.e., Ras, Myc) and loss of function of negative growth regulators (suppressor oncogenes, i.e. Rb, p53) leads to aberrant cell cycle control including loss of normal checkpoint responses.

Increased Action of Positive Growth Regulators: Oncogenes

Q. Define proto-oncogenes and oncogene. List the different products of oncogenes.

Q. Write short note on oncogene.

Q. Describe the mechanism of activation of oncogene giving suitable examples.

Proto-oncogenes are normal cellular genes, which encode a number of nuclear proteins that **regulate normal cell proliferation, differentiation and survival**. Proto-oncogenes have multiple roles, but all act at some level in signaling pathways involved in proliferation of cells.

Proto-oncogene: Normal cellular genes whose products are involved in normal cell growth and repair process.

Oncogenes and oncoproteins: Mutation of normal cellular genes known as proto-oncogenes produces genes that lead to tumor formation and these **altered/mutated versions of proto-oncogenes are termed as oncogenes**. These oncogenes promote autonomous cell growth in cancer cells. These oncogenes usually produce increased encoded **gene product called oncoprotein** and cause tumors. These mutations are called as “**gain-of-function**,” mutations because they can **transform cells even in the presence of a normal copy of the same gene**. Thus, oncogenes are dominant over their normal counterparts and behave as dominant genes.

- **Oncogenes** have the ability to **promote cell growth in the absence** of external normal growth-promoting/mitogenic signals/stimuli.
- **Products** of oncogenes → are called **oncoproteins**, which resemble the normal products of proto-oncogenes.
- **Oncoprotein production is not under normal regulatory control** → cells proliferate without the usual requirement for external signals and are freed from checkpoints → **growth becomes autonomous**. **Oncoproteins act like accelerators that speed the replication of cells and their DNA**. In contrast, tumor suppressors act as brakes that slow or arrest this process.

Oncogene: Mutated or overexpressed version of **proto-oncogene**. They function autonomously without requiring normal growth-promoting signals. They are not under normal regulatory control.

Oncoproteins: Products of oncogenes that cause uncontrolled proliferation of cells by several mechanisms.

Classification of oncogenes: Oncogenes can be classified (Table 7.10) according to the function of gene product (oncoprotein) as:

- Growth factors
- Growth factor receptors
- Signal transduction proteins
- DNA-binding nuclear regulatory proteins/transcription factors
- Cell cycle regulators.

Growth Factor Oncoproteins

- Normal cell proliferation requires stimulation by growth factors.
- Neoplasm may be associated with **excessive production of growth factors** by oncogenes.
- **Action of growth factor oncoprotein:** These oncoproteins may act by one of the two ways: (1) Paracrine or (2) autocrine action.
- Example: In **glioblastomas** (malignant glial cell tumors) the tumor cells itself secrete **excess platelet-derived growth factor (PDGF)** and express **PDGF receptor tyrosine kinases**.

Growth factor oncoproteins—PDGF: Excessively produced in glioblastoma.

Growth Factor Receptor Oncoproteins

Normally, when the growth factor binds to the growth factor receptors, it produces transient dimerization (activity). **Constitutive (unrestrained) dimerization** of growth factor receptors → produces continuous mitogenic signals to the cell, **even in the absence of the growth factor**.

Mechanism of activation of receptor tyrosine kinases: Growth factor receptors can be constitutively activated in tumors by multiple mechanisms, including point mutations, gene rearrangements and gene amplifications.

1. **Point mutation:** *ERBB1* point mutation in a subset of lung adenocarcinomas.
2. **Gene amplification:** *ERBB2* (also called *HER-2/Neu*) gene is amplified in certain **breast cancers**.
3. **Gene rearrangements:** They activate other receptor tyrosine kinases (e.g. tyrosine kinase *ALK*). Example: A deletion on chromosome 5 results in fusion of part of the *ALK* gene with part of another gene called *EML4* in a subset of lung adenocarcinomas resulting in *EML4-ALK* fusion gene.

Growth factor receptor oncogene-*ERBB2* (Her-2/Neu) is overexpressed in:

- Breast carcinoma
- Non-small cell lung carcinoma
- Ovarian carcinoma

TABLE 7.10: Categories of oncogenes and examples of associated tumors

Category of oncogene	Proto-oncogene	Examples of associated tumors
Growth Factors		
PDGF- β chain	<i>PDGFB</i>	Astrocytoma
Fibroblast growth factors	<i>HST1</i>	Osteosarcoma
	<i>FGF3</i>	Cancer of stomach, bladder, breast and melanoma
HGF	<i>HGF</i>	Hepatocellular carcinomas, thyroid cancer
Growth Factor Receptors		
EGF-receptor family	<i>ERBB1 (EGFR)</i>	Adenocarcinoma of lung
ALK receptor	<i>ALK</i>	Adenocarcinoma of lung certain lymphomas, neuroblastoma
Receptor for neurotrophic factors	<i>RET</i>	Multiple endocrine neoplasia 2A and B, familial medullary carcinoma thyroid
Receptor for KIT ligand	<i>KIT</i>	Gastrointestinal stromal tumors, seminomas, leukemias
FMS-like tyrosine kinase 3	<i>FLT3</i>	Leukemia
PDGF receptor	<i>PDGFRB</i>	Gliomas, leukemias
Signal Transduction Proteins		
GTP-binding (G) proteins	<i>KRAS</i>	Tumors of colon, lung and pancreas
	<i>HRAS</i>	Tumors of bladder and kidney
	<i>NRAS</i>	Melanomas, hematologic malignancies
RAS signal transduction	<i>BRAF</i>	Melanomas, leukemias, colon carcinoma
Nonreceptor tyrosine kinase	<i>ABL</i>	Chronic myelogenous leukemia, acute lymphoblastic leukemia
JAK/STAT signal transduction	<i>JAK2</i>	Myeloproliferative neoplasms, acute lymphoblastic leukemia
Notch signal transduction	<i>NOTCH1</i>	Leukemias, lymphomas, breast carcinoma
Nuclear Regulatory Proteins/Transcription Factors		
Transcriptional activators	<i>MYC</i>	Burkitt lymphoma
	<i>NMYC</i>	Neuroblastoma
Cell Cycle Regulators		
Cyclins	<i>CCND1 (Cyclin D1)</i>	Mantle cell lymphoma, multiple myeloma, breast and esophageal cancers
Cyclin-dependent kinase	<i>CDK4</i>	Glioblastoma, melanoma, sarcoma

Proto-oncogenes: Discovered by Harold Varmus and Michael Bishop.

Signal-transducing Oncoproteins

GTP-binding (G) Proteins: Normal RAS Cycle (Fig. 7.20)

- RAS proteins (product of *RAS* gene) are attached to the cytoplasmic aspect of the plasma membrane by farnesyl (also the endoplasmic reticulum and Golgi membranes).
- Normally, RAS proteins orderly cycles between inactive state [RAS proteins bound to guanosine diphosphate

(GDP)] and active signal-transmitting state (RAS is bound to GTP).

- Stimulation of cells by growth factors activate RAS. The active GTP state is short-lived because an enzyme GTPase hydrolyzes GTP \rightarrow GDP.
- Activated RAS stimulates downstream regulators of cell proliferation by two pathways: (1) RAF/ERK/MAP kinase pathway and (2) PI3 kinase/AKT pathway, which in turn send the signal to the nucleus resulting in cell proliferation.

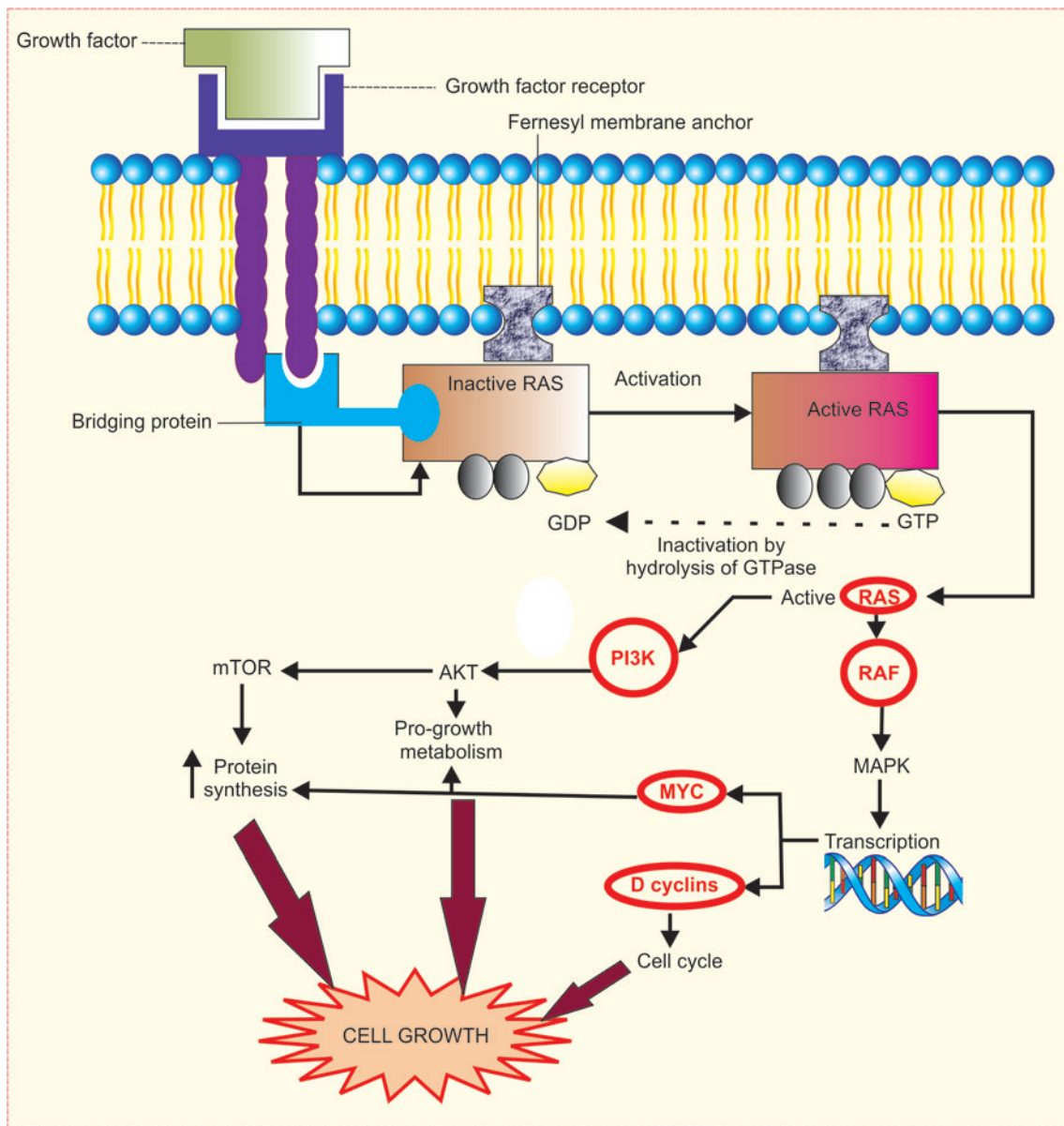


Fig. 7.20: Normal RAS cycle and growth factor signaling pathways in cancer. RAS is anchored to the cell membrane by the farnesyl moiety and is essential for its action. When a growth factor binds to growth factor receptor, inactive (GDP bound) RAS become activated to a GTP bound state. The active GTP state is short-lived because an enzyme GTPase hydrolyses GTP to GDP. Activated RAS in turn transduces proliferative signals to the nucleus. Growth factor receptors, RAS, PI3K, MYC and D cyclins are oncoproteins. These are activated by mutations in various cancers

RAS oncogenes:

Q. Write short note on RAS oncogene.

RAS proto-oncogene can be converted to RAS oncogene by mutation (mainly point mutation). The mutated RAS is trapped in its activated GTP-bound form → results in continuous proliferation of cells.

- **Tumors with RAS mutations:** Human genome contains three types of RAS genes.

- **KRAS:** Mutation in adenocarcinomas of colon, lung and pancreas
- **HRAS:** Mutations in bladder and kidney tumors
- **NRAS:** Mutations in melanoma, hematopoietic tumors.

RAS family of oncoproteins is an example of signal-transducing proteins

Point mutation of RAS genes is the most common, frequent and dominant cause of human tumors.

BRAF mutations: BRAF is a serine/threonine protein kinase belonging to MAPK family. Similar to activating RAS mutations, activating point mutations in BRAF activate transcription factors. Mutations of BRAF is seen in hairy cell leukemias, melanomas, benign nevi and in few cancers of colon. Activation of the PI3K by point mutations also occurs in many cancers.

Nonreceptor tyrosine kinase-ABL

ABL is a non-receptor-associated tyrosine kinase which functions as signal transduction molecule. *ABL* is a proto-oncogene and has a tyrosine kinase activity.

- In **chronic myelogenous leukemia**, *ABL* proto-oncogene from chromosome 9 joins the *BCR* on chromosome 22 (See Fig. 7.19). It produces a new **chimeric (fusion) gene called *BCR-ABL***, thus converting *ABL* proto-oncogene into oncogene → oncoprotein (e.g. p210) → causes cell division and inhibition of apoptosis.
- Point mutation of ABL: In acute lymphoblastic leukemia.

DNA-binding Nuclear Regulatory Proteins (Transcription Factors)

Q. Write short note on MYC oncogene.

- All signal transduction pathways stimulate nuclear transcription factors, which bind DNA and regulate transcription of genes.
- Transcription is a process in which RNA is synthesized from DNA. **Transcription factors stimulate growth-promoting genes which activate cell cycle.**
- Tumor may develop due to mutations of transcription genes. The mutation results in oncogenes like *MYC*, *MYB*, *JUN*, *FOS* and *REL*, whose products (oncoproteins) are transcription factors that regulate the expression of growth-promoting genes, such as cyclins. ***MYC* is most commonly involved in human tumors.**

MYC Oncogene

- ***MYC* proto-oncogene is expressed in all cells during normal cell proliferation. *MYC* activates the expression of several genes involved in cell growth.** These include D cyclins (involved in cell cycle progression) and rRNA genes and rRNA processing (increases the assembly of ribosomes needed for protein synthesis). It also upregulates gene expression that results in metabolic reprogramming and the Warburg effect. Because of their several effects, *MYC* is considered a **master transcriptional regulator of cell growth**. Example, rapid growth in Burkitt lymphoma has chromosomal translocation involving *MYC* and has highest level of *MYC*.

- **In few tumors, *MYC* upregulates expression of telomerase (responsible for unlimited replication-the immortalization of cancer cells).**
- ***MYC* is one of transcription factor which can reprogram somatic cells into pluripotent stem cells** thereby leading to immortalization of cancer cells.

Mechanism of deregulation of MYC

1. **Genetic alterations of *MYC* itself** causes overexpression of the *MYC* protein.
2. ***MYC* translocations:** E.g. *C-MYC* in Burkitt lymphoma
3. ***MYC* is amplification:** E.g. some carcinoma of breast, colon, lung, etc. Functionally identical *N-MYC* gene amplification in neuroblastomas and *L-MYC* genes amplification in small cell cancers of the lung.
4. **Oncogenic mutations of upstream signaling pathways:** These may cause increased levels of *MYC* protein by increasing *MYC* transcription, increasing *MYC* mRNA translation, and/or stabilizing *MYC* protein.

N-MYC amplification is associated with: Neuroblastoma.

Cyclins and Cyclin-dependent Kinases (CDKs)

Transition from G₁ to S phase of the cell cycle is controlled by: Cyclin D.

All growth-promoting stimuli, finally, causes the entry of quiescent cells into the cell cycle. The cell cycle is regulated by cyclins and cyclin-dependent kinases.

Role of Cyclins in Normal Cell Cycle

Q. Write short note on role of cyclins in the cell cycle.

- The various phases of the cell cycle are regulated by **cyclins** (named so because of cyclic nature of their production and degradation) and **cyclin-dependent kinases (CDKs)**.
- The CDK-cyclin complexes phosphorylate essential proteins which activate the cell cycle, following which the cyclin levels decline quickly.
- Of the several (more than 15) distinct set of cyclins; **cyclins D, E, A and B** are important which appear sequentially (one after another) during the cell cycle.
- While cyclins and CDKs drive the cell cycle, **negative control over the cell cycle** is achieved by **silencing the CDKs by their inhibitors (CKIs)**.

Role of cyclins and CDKs in regulating cell cycle are mentioned in Table 7.11.

Cyclin D is the first cyclin to increase in the cell cycle.

Tumor suppressor gene *p53* induces cell cycle arrest at: G_1 to S phase

Transition from G_2 to M phase of the cell cycle is controlled by: Cyclin B.

Fixed time is required for steps of cell cycle: S and M phase

TABLE 7.11: Role of cyclins and cyclin-dependent kinases in regulation of cell cycle

Type of cyclin and cyclin-dependent kinase (CDK)	Phase of cell cycle
Cyclin D/CDK4	Regulation of transition from G_1 to S phase by phosphorylation of RB protein
Cyclin D/CDK6	
Cyclin E/CDK2	
Cyclin A/CDK2	Active in S phase
Cyclin A/CDK1	
Cyclin B/CDK1	Essential for transition from G_2 to M phase

Cellular content of DNA is doubled during S phase of the cell cycle.

Correct sequence of cell cycle is: G_0 - G_1 - S - G_2 - M .

Alteration in Cell Cycle Control Proteins in Cancer

Cell cycle has two main checkpoints: (1) At the G_1/S transition and (2) at the G_2/M transition. Both are tightly regulated by a balance of growth promoting and growth suppressing factors and also by sensors of DNA damage. If DNA-damage sensors are activated, they arrest the progression of cell cycle and allow the repair of DNA by DNA-repair genes. If cell damage cannot be repaired, these sensors initiate apoptosis of the cells. Defects in the G_1/S checkpoint are more important in cancer than G_2/M checkpoint. The major cancer-associated mutations that affect the G_1/S checkpoint are mainly divided into two groups.

- Gain-of-function mutations in D cyclin genes and CDK4:** These oncogenes promote G_1/S progression.
 - D cyclin genes:** These include D1, D2 and D3 and they undergo mutations in cancer by chromosomal translocations (e.g. lymphomas) and gene amplification (e.g. solid tumors).
 - Amplification of the CDK4 gene:** It is observed in melanomas, sarcomas and glioblastomas.
- Loss-of-function mutations in tumor suppressor genes that inhibit G_1/S progression:** CDKIs which inhibit cyclin D/CDK complexes are mutated or silenced in some malignant tumors. Examples: Deletion or inactivation of

CDKN2A (*p16*) in pancreatic carcinomas, glioblastomas, esophageal carcinoma, acute lymphoblastic leukemias, and non-small-cell lung carcinomas. Apart from this, two most important tumor suppressor genes, *RB* and *TP53*, encode proteins which prevent G_1/S progression.

Above mutations with activation of cyclin D or CDK4 and mutational inactivation of CDK inhibitors cause proliferation of cells by hyperphosphorylation and inactivation of RB. This causes release of E2F transcription factors which leads to the expression of genes required for progression from G_1 to S phase.

Loss of Function of Negative Growth Regulators (Tumor Suppressor Genes)

Note: Gene symbols are italicized but not their protein products.

Q. Write short note on tumor suppressor genes and cancers produced by their mutations.

Tumor suppressor is a protein or gene, is associated with suppression of any of the various hallmarks of cancer. As discussed earlier, oncogenes stimulate proliferation of cells, whereas the products of most tumor suppressor genes apply brakes and prevent uncontrolled cell proliferation. Tumor suppressor proteins form a network of checkpoints and act as negative growth regulators. They prevent uncontrolled growth. Abnormalities in these tumor genes lead to loss of function of negative growth regulators i.e. failure of growth inhibition. So, a second mechanism of carcinogenesis results from failure of negative growth regulator (growth inhibition), due to deficiency of normal tumor suppressor genes and their products.

General Characteristic Features of Tumor Suppressor Genes

Q. Define cancer suppressor gene and cancers produced by their mutations.

- Mechanism of action:** Most tumor suppressors inhibit cell growth through one or other mechanism. Mutations that affect tumor suppressor genes usually cause a "loss-of-function."
 - Apply brakes to cell proliferation:** Many tumor suppressors (e.g. two important tumor suppressor genes *RB* and *p53*) are part of a regulatory network and they apply the brakes on cell cycle progression and DNA replication. They recognize genotoxic stress from any source and prevent proliferation of these cells. Thus, an oncogene in normal cells with intact tumor suppressor genes may result in quiescence, or permanent arrest of cell cycle (oncogene-induced

senescence), rather than uncontrolled proliferation. These cells may ultimately undergo apoptosis. Abnormalities in these genes lead to failure of growth inhibition.

- **Other mechanisms:** Some tumor suppressors prevent cellular transformation through other mechanisms. These include **by altering cell metabolism** (e.g. the serine-threonine kinase STK11) or **by maintaining genomic stability** (e.g. the DNA repair factors BRCA1 and BRCA2).
2. **Mutations of tumor suppressor genes may be hereditary and spontaneous.**
 3. **Loss of heterozygosity:**
 - Usually, for tumor to develop, **both normal alleles of tumor suppressor genes must be inactivated (damaged/mutated)**.
 - Heterozygous state (one allele normal and other allele inactive) is sufficient to protect against cancer.
 - Cancer develops when the cell loses heterozygosity (known as loss of heterozygosity—LOH) for the normal tumor suppressor gene by **deletion or somatic mutation**. Tumor can develop when the cell becomes homozygous (both alleles are inactive) for the mutant allele. Thus, mutated tumor suppressor genes usually behave in a recessive fashion. However, sometimes, loss of a single allele of a tumor suppressor gene can lead to cell proliferation. When loss of gene function is caused by damage to a single allele, it is called haploinsufficiency.
 4. **Groups of tumor suppressor genes:** (a) Governors and (b) guardians.
 - **Governor gene** mutations → remove the brake for cellular proliferation → neoplasia, e.g. *RB* gene.
 - **Guardian genes** sense the genomic damage and prevents proliferation of cells with genetic damage or if damage is too severe to be repaired → induces apoptosis e.g. *p53*.

Tumor suppressor genes: Protect the cells against unregulated proliferation.

Tumor suppressor gene: For tumor formation both copies of genes must be inactivated.

Retinoblastoma Gene (*RB* Gene)

Q. Write short note on Knudson's two-hit hypothesis.

RB (*RB1*) gene was the first discovered tumor suppressor gene, which is present on chromosome locus 13q14.

Inactivation of *RB* gene was found in retinoblastoma, which is a rare malignant childhood tumor derived from the retina. Retinoblastoma may occur either as a hereditary or sporadic form.

Knudson's two-hit hypothesis of oncogenesis: It explains the inherited and sporadic occurrence of an identical tumor. According to Knudson's hypothesis:

- **Two mutations (hits)**, involving **both alleles** of tumor suppressor gene **are required to produce the tumor**.
- **In familial cases**, one mutation (**first hit**) takes place in the germ line and second hit after birth.
- **In sporadic cases** both mutations (**two hits**) develop after birth.

RB gene: First discovered tumor suppressor gene.

RB gene is present on chromosome 13q14.

RB gene product is *RB* protein.

RB Gene and Retinoblastoma

1. **Familial/hereditary retinoblastoma:** It constitutes about 40% of retinoblastoma and two hits occurs as follows:
 - **First hit:** Affected **children inherit** cells with **one defective copy** (mutated allele) of the *RB* gene in the **germ line** (one hit) and one normal copy of *RB* gene (the child is heterozygous at the *RB* locus). The product of normal *RB* gene is sufficient to prevent tumor.
 - **Second hit:** **Retinoblastoma develops** when the remaining normal *RB* allele is inactivated (mutated) due to **spontaneous somatic mutation** (second hit). Because only a single somatic mutation is sufficient for loss of *RB* function in familial retinoblastoma, (it is **transmitted as an autosomal dominant trait**).

Patients with **familial retinoblastoma** have also **increased risk** of developing **osteosarcoma** and other soft-tissue sarcomas.

2. **Sporadic retinoblastoma:** It forms about 60% of cases. The child has two normal *RB* alleles in all somatic cells. To develop retinoblastoma, **both normal *RB* alleles must undergo mutation** and it needs **two hits**.

Patients with *RB* mutations have increased risk:

1. Retinoblastoma
2. Osteosarcoma
3. Soft-tissue sarcomas.

Functions of the RB Gene (Fig. 7.21)

Q. Write short note on role of RB in the cell cycle.

Q. Function of retinoblastoma gene.

RB gene is governor of cell cycle and plays a key role in **regulating the cell cycle** and also controls cellular differentiation.

Normal cell cycle has two gaps:

1. **Gap 1 (G_1)** between mitosis (M) and DNA replication (S). Gap 1 is **very important checkpoint**, because once the cells cross this checkpoint they are compelled to complete mitosis. In G_1 phase, **signals determine whether the cell should enter the cell cycle, exit the cell cycle either temporarily (known as quiescence), or permanently (known as senescence). RB plays a key role in this decision process.**

2. **Gap 2 (G_2)** between DNA replication (S) and mitosis (M).

- **State of RB gene product:** **RB gene product** is a DNA-binding protein expressed in all cells. It is present either in an **active hypophosphorylated state** (in quiescent cells) or **inactive hyperphosphorylated state** (in cells passing through the G_1/S cell cycle phase).
- **Active RB gene regulates G_1/S checkpoint of cell cycle:** Cell cycle is tightly controlled by cyclins and cyclin-dependent kinases (CDKs), which form cyclin-CDK complexes.
 - Before DNA replication, the cell must pass through G_1/S check which is regulated by **RB**.
 - **Initiation** of DNA replication (S phase) requires activation of cyclins D/CDK4, cyclin D/CDK6 and cyclin E/CDK2 complexes. High levels of these complexes lead to hyperphosphorylation and inhibition of RB. This releases E2F transcription

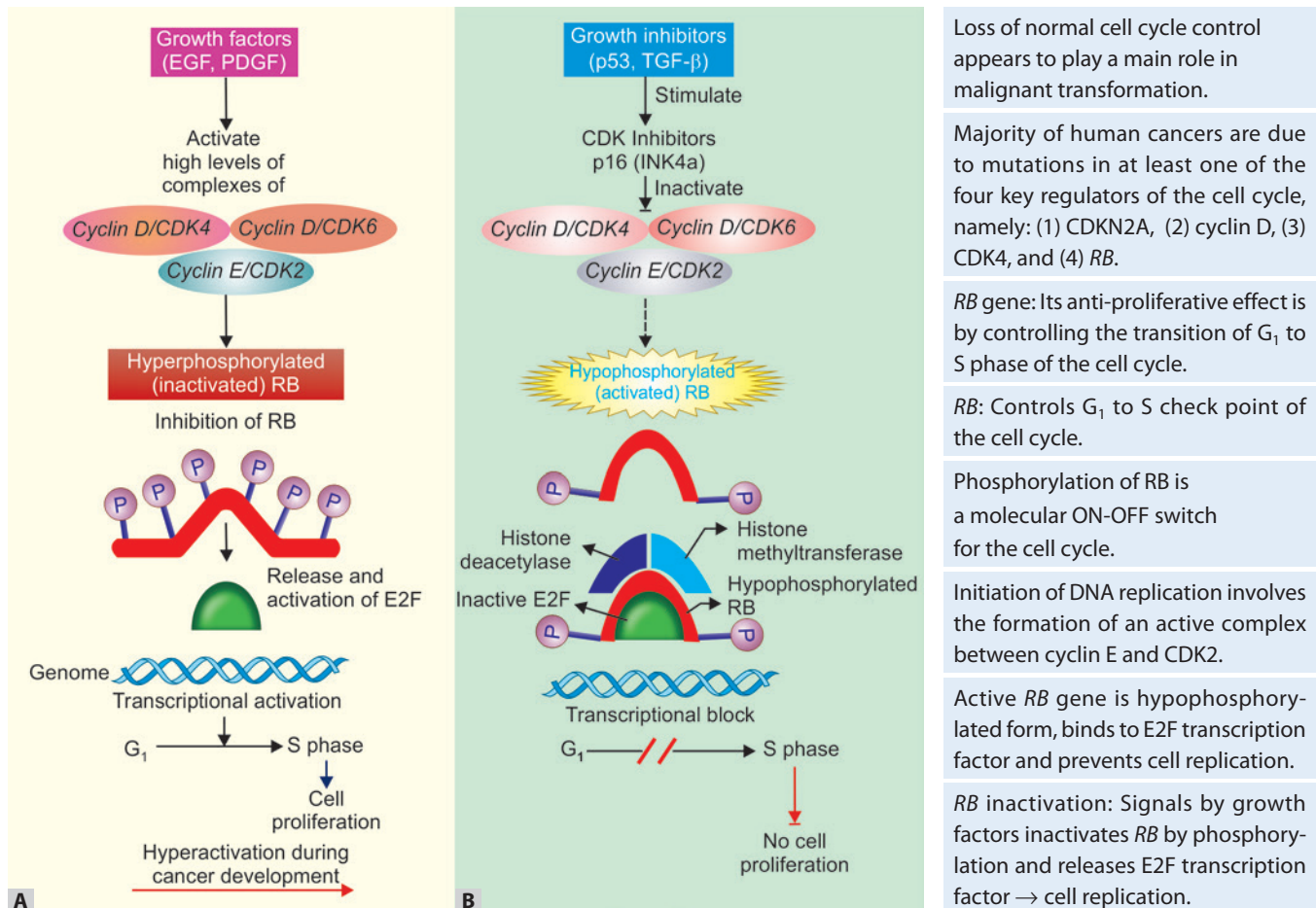


Fig. 7.21: Function of *RB* in regulating the G_1 -S checkpoint of the cell cycle: (A) When *RB* is phosphorylated by the cyclin D-CDK4/6 complexes, it releases E2F. The latter then activates transcription of S-phase genes; (B) Hypophosphorylated active *RB* combines with the E2F transcription factors along with histone deacetylases and histone methyltransferases, and inhibits progression from G_1 -S phase of cell cycle

factors which causes the expression of genes that are required for progression cell from G₁ to S phase.

- **RB blocks E2F-mediated transcription:** During early G₁ phase, **active hypophosphorylated** RB binds to E2F family of transcription factors. Two methods of blocking transcription are:
 1. **Sequesters E2F** and prevent it from interacting with other transcription activators.
 2. Recruits two enzymes (histone deacetylases and histone methyltransferases) that **block the transcription**.

Inactivation of RB gene: Growth factor (mitogenic) signaling → upregulate the activity of the CDK/cyclin complexes → conversion of active hypophosphorylated RB into inactive hyperphosphorylated RB.

- **Consequence of inactivation of RB gene:** Inactivation of RB release the break and frees the transcription factor E2F from RB → DNA replication → progression of cell cycle.

Reactivation of RB gene: During M phase phosphate groups are removed from hyperphosphorylated RB by cellular phosphatases → regeneration of active hypophosphorylated RB.

Method of Inactivation of RB Gene and Associated Tumors

1. **Loss-of-function mutations involving both RB alleles.** It may be:
 - **Germ-line mutation**, e.g. in **retinoblastomas** and **osteosarcomas**.
 - **Acquired mutation**, e.g. in **glioblastomas**, **small-cell carcinomas of lung**, breast cancers and bladder carcinomas.

Most common secondary malignancy in a patient with retinoblastoma is: Osteosarcoma

2. **Other mechanism:** The active hypophosphorylated RB state may be shifted to an inactive hyperphosphorylated RB state. This may be due to (1) gain-of-function mutations that upregulate CDK/cyclin D activity or (2) by loss-of-function mutations that abolish/cancel the activity of CDK inhibitors (p16/INK4a).
3. **Viral oncoproteins that bind and inhibit RB (E7 protein of HPV)** may occur even without RB mutation. Example: E7 protein of human papillomavirus (HPV) bind to the hypophosphorylated RB → prevents binding of RB protein with E2F transcription factors → free E2F causes progression of cell cycle → cervical carcinomas (Fig. 7.27).

In the majority of cancers at least one of four key regulators of the cell cycle, namely (1) p16/INK4a, (2) cyclin D, (3) CDK4, or (4) RB is dysregulated.

DNA oncogenic viruses (e.g. HPV) encode proteins (e.g. E7) that bind to RB → blocks RB function.

TP53 Gene (Guardian of the Genome)

TP53 gene product is protein p53.

Q. Write short note on p53/TP53 gene and its role in neoplasia.

TP53 is a **tumor suppressor gene located on small arm of chromosome 17(17p13.1)**. Its protein product p53 is present in almost all normal tissues. Loss-of-function mutations in **TP53** is the most common mutations observed in more than 50% of cancers. **TP53** mutations occur at variable frequency with almost every type of cancer, including the three leading causes of cancer death namely carcinomas of the lung, colon and breast.

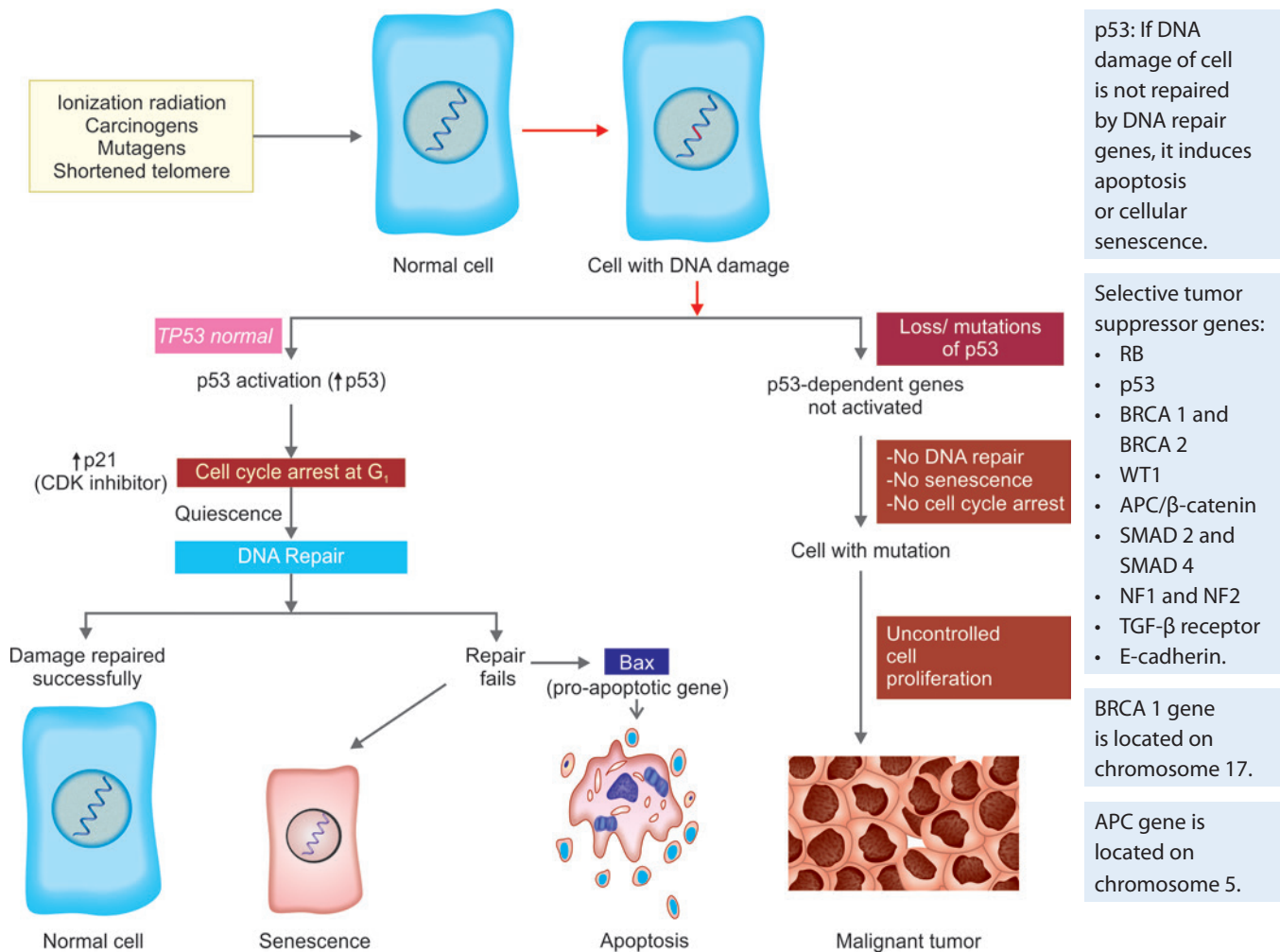
Functions of p53 (Fig. 7.22)

Guardian of the genome: It functions as **critical gatekeeper genes**. It plays **main role in maintaining the integrity of the genome** and thus known as **guardian of the genome** or “**molecular policeman**.”

p53: Guardian of the genome.

Role of TP53: **TP53** has critical role in the prevention of cancer development and p53 serves as focal point of large network of signals which sense cellular stress, DNA damage, shortened telomeres, hypoxia and stress caused due to increased pro-growth signaling (e.g. cells with mutations in **RAS** and **MYC** genes).

- **In nonstressed, healthy/normal cells, p53 is maintained at low levels** by MDM2 (murine double minute). MDM2 is an E3 ubiquitin (Ub) ligase that conjugates p53 to Ub and degrades p53.
- **In stressed cells**, p53 is released from the inhibitory effects of MDM2 and **p53 becomes activated**. Activation of p53 may occur through two mechanisms that depend on the nature of the stress.
 - **DNA damage and hypoxia:** Stress due to DNA damage or hypoxia activates two related protein kinases, namely 1) ataxia-telangiectasia mutated (ATM) and 2) ataxia-telangiectasia and Rad3 related (ATR). ATM gene was first identified as the germ-line mutation in patients with ataxia-telangiectasia (inability to repair certain kinds of DNA damage, and have increased incidence of cancer). Activated ATM and ATR stimulate the phosphorylation of p53 and MDM2. This disrupts the binding and degradation of p53 by MDM2 and leads to activation and accumulation of p53.
 - **Oncogenic stress:** It may be induced by activation of oncoproteins such as RAS. These stresses produce



p53 gene located on small arm of chromosome 17(17p13).

p53: DNA oncogenic viruses (e.g. HPV) encode proteins that bind to p53 and blocks its function.

Fig. 7.22: Role of *p53* in maintaining the integrity of the genome. DNA damage activates normal *p53* and arrests the cell cycle in G₁ and induces repair of DNA. Successful repair of DNA allows cells to proceed with the cell cycle; if DNA repair fails, *p53* triggers either apoptosis or senescence. In cells with loss or mutations of *p53*, DNA damage does not induce cell cycle arrest or DNA repair or senescence, and cells with mutation proliferate to form malignant neoplasms

sustained signaling via pro-growth pathways (e.g. MAPK and PI3K/AKT pathways). These signals produce cellular stress and lead to increased expression of p14/ARF (encoded by the *CDKN2A* tumor suppressor gene). p14/ARF binds MDM2 and releases p53 and resulting in raised p53 levels in the cell.

Prevention of neoplastic transformation: Activated p53 prevents neoplastic transformation of cell by three interconnected mechanisms:

1. **Transient/temporary p53-induced cell cycle arrest:** If there is **damage to DNA**, transient, rapid cell cycle arrest occurs late in the G₁ phase. It is brought-out partly by p53-dependent transcription of the *CDKN1A* gene (encodes the CDK inhibitor p21). p21 in turn

inhibits CDK4/D cyclin complexes and maintain RB in an active, hypophosphorylated state. This blocks the progression of cells from G₁ phase to S phase. This cell cycle arrest gives the cells time to repair DNA damage. **If DNA damage is repaired**, the signals that caused stabilization/ activation of p53 disappears. This results in **fall in the levels of p53 and releases the block in cell cycle** and return of cells to a normal state.

2. **p53-induced senescence (permanent cell cycle arrest):** Senescence is defined as a state of permanent cell cycle arrest. Senescence may be stimulated in response to different types of stresses (e.g. unopposed oncogene signaling, hypoxia and shortened telomeres). The senescent cells are prevented from forming tumors.

3. **p53-induced apoptosis (programmed cell death):** Cells with irreversible DNA damage undergo p53-induced apoptosis and is the protective mechanism against development of cancer. p53 stimulates transcription of several pro-apoptotic genes (e.g. *BAX* and *PUMA*) resulting in apoptosis of cells via the intrinsic (mitochondrial) pathway.

Method of inactivation of TP53 gene and associated tumors: Most cancers have defect in TP53 gene.

1. **Acquired loss-of-function mutation in both (biallelic) TP53 alleles** in somatic cells is most common.
2. **Germ-line mutations in one TP53 allele:** It is less common. Individuals may inherit one mutated/defective TP53 allele and one additional “hit” in the other normal TP53 allele will produce malignant tumors. For example: Li-Fraumeni syndrome has germ-line mutations in one TP53 and these individuals usually develop cancer at younger age, have 25-fold greater chance of developing a malignant tumor by age 50 and are more prone to develop multiple primary tumors of varying types.
3. **Mutations of proteins that regulate p53 function:** TP53 encodes the protein p53, the function of which is tightly regulated at several levels by other proteins. Thus, many tumors without TP53 mutations have mutations of proteins that regulate p53 function. For example: MDM2 and related proteins of the MDM2 (enzyme that ubiquitinylates p53) family degrade p53 leading to a functional deficiency of p53. These proteins are frequently overexpressed in cancers with normal TP53 alleles.
4. **Blocking of p53 function:** Similar to RB, the transforming proteins of many DNA viruses bind and degrade p53 even without mutation in p53. For example, viral oncoprotein E6 of high-risk human papillomaviruses (HPVs) promote p53 degradation and cause cervical carcinoma and a subset of squamous cell carcinomas of the head and neck.

Consequences of Loss of p53 Function

- DNA damage goes unrepaired.
- Driver mutations accumulate in oncogenes and other cancer genes.
- Cell blindly follows a dangerous path leading to malignant transformation.

Therapeutic Implications of TP53

- **Wild type versus mutated TP53:** Irradiation and chemotherapy used for the treatment of cancer, mediate their effects by causing damage to the DNA and producing apoptosis of tumor cells. Tumors with wild type TP53 (wild type refers to the most common form

or phenotype in nature) alleles are more susceptible for apoptosis than tumors with mutated TP53 alleles. For example: Childhood acute lymphoblastic leukemias which have wild type TP53 alleles respond to radio and chemotherapy; whereas lung cancers and colorectal cancers with mutated TP53 allele, are relatively resistant to chemotherapy and irradiation.

- **Consequences of mutated TP53:** Tumor cells with mutated p53 have a tendency to acquire additional mutations at a high rate and are resistant to any mono/single therapy (radiation/conventional chemotherapy/molecularly targeted therapy).

Other p53 family members: These include p63 and p73. p53 is universally expressed, whereas p63 and p73 show more tissue specificity. For example, p63 is required for the differentiation of stratified squamous epithelium and p73 has powerful pro-apoptotic effects after DNA damage produced by chemotherapeutic drugs.

Location, function and tumors associated with few selected tumor suppressor genes are presented in Table 7.12.

Non-mutated TP53 is also called as the ‘wild type’ of TP53 gene and is associated with reduced risk of cancers.

Consequences of loss of function of p53:

1. DNA damage remains unrepaired.
2. Mutations accumulate in dividing cells.
3. Cell undergoes malignant transformation.

p53 is activated whenever there is damage to cellular DNA. Activated p53 causes G₁ arrest of a cell allowing time for the DNA repair by DNA-repair genes.

p53 can be inactivated by oncogenic viruses, such as HPV.

p53: tumors with normal p53 respond better to chemotherapy and radiotherapy than those with mutated p53. Tumors with p53 mutations are relatively resistant to treatment.

Activated p53 controls genes involved in cell cycle, DNA repair, cellular senescence and apoptosis.

p53 directs the cell with unrepaired DNA to undergo death by apoptosis.

p53 induces cell arrest at: G₁-S phase.

Altered Cellular Metabolism in Cancer Cells (Warburg Effect)

- Cancer cells have different needs than their normal counterpart. Their proliferative rate generally exceed that of normal cells. Cancer cells must quickly synthesize the structural components (e.g. protein, lipid, etc.) that are required for rapid cell growth (that is to sustain their mitotic activity).
- **With adequate oxygen supply,** cancer cells undergo a metabolic switch to aerobic glycolysis. They develop a distinctive form of cellular metabolism characterized

TABLE 7.12: Few tumor suppressor genes and associated familial syndromes and sporadic cancers**Q. Write short note on familial syndromes/ inherited cancers**

Gene (protein)	Function	Familial Syndromes	Sporadic Cancers
Inhibitors of mitogenic signaling pathways			
APC (Adenomatous polyposis coli)	Inhibits WNT signaling	Familial colonic polyps and carcinomas	Carcinomas of stomach, colon, pancreas; melanoma
NF1 (Neurofibromin-1)	Inhibits RAS/MAPK signaling	Neurofibromatosis type 1	Neuroblastoma
PTEN (Phosphatase and tensin homologue)	Inhibits of PI3K/AKT signaling	Cowden syndrome	Carcinomas and lymphoid tumors
SMAD2, SMAD4 (SMAD2, SMAD4)	TGF- β signaling pathway	Juvenile polyposis	Carcinoma of colon and pancreas
Inhibitors of cell cycle progression			
RB (Retinoblastoma protein)	G ₁ /S transition during cell cycle	Familial retinoblastoma syndrome	Retinoblastoma; osteosarcoma, carcinomas of breast, colon, lung
Inhibitors of “pro-growth” programs of metabolism and angiogenesis			
VHL (Von Hippel Lindau protein)	Hypoxia-induced transcription factors (e.g. HIF1 α)	Von Hippel Lindau syndrome	Renal cell carcinoma
Inhibitors of invasion and metastasis			
CDH1 (E-cadherin)	Cell adhesion, inhibition of cell motility	Familial gastric cancer	Gastric carcinoma, lobular carcinoma of breast
Enablers of genomic stability			
TP53 (p53 protein)	Cell cycle arrest and apoptosis in response to DNA damage	Li-Fraumeni syndrome	Majority of cancers
DNA repair factors			
BRCA1 (Breast cancer-1), BRCA2 (breast cancer-2)	Repair of double-stranded breaks in DNA	Familial carcinoma of breast and ovary; carcinomas of male breast; chronic lymphocytic leukemia (BRCA2)	Rare
Unknown mechanisms			
WT1 (Wilms tumor-1)	Transcription factor	Familial Wilms tumor	Wilms tumor, certain leukemias

by increased amount of glucose uptake and increased conversion of glucose to lactose (fermentation) via the glycolytic pathway. This *aerobic glycolysis* is called the *Warburg effect*. It was described in 1930 by Otto Warburg and is not cancer specific, but observed in growing cells and it becomes “fixed” in cancer cells.

- **The aerobic glycolysis provides metabolic intermediates that are needed for the synthesis of cellular components in rapidly dividing tumor cells.** This cannot be met with normal mitochondrial oxidative phosphorylation.
- **Clinical utility:** The “glucose-hunger” of tumors is made use for visualization of tumors in **positron emission tomography (PET) scanning**. In PET scanning, patients are injected with ¹⁸F-fluorodeoxyglucose (a non-metabolizable derivative of glucose) which is preferentially taken up into tumor cells (and also actively dividing normal cells, e.g. bone marrow cells). **Most tumors are PET-positive**, and markedly positive are the rapidly growing tumors.

Loss of Normal Apoptosis Pathways

Apoptosis is a programmed cell death and is **one of the normal protective mechanism by which a cell with DNA damage (mutation) undergo cell death**. Many types of signals such as DNA damage, potent oncoproteins such as MYC, and loss of adhesion to the basement membrane (termed anoikis), can initiate apoptosis. Mutations in the genes that regulate apoptosis may result in accumulation of neoplastic cells.

- Abnormalities of *apoptosis-regulating genes* may result in less death and increased survival of the cells. These abnormalities may be gain-of-function mutations in genes whose products suppress apoptosis and loss-of-function mutations in genes whose products promote cell death. The *apoptosis-regulating genes* can behave as **proto-oncogenes** (loss of one copy is enough) or **tumor suppressor genes** (loss of both copies required).

Pathways of Apoptosis (Fig. 1.25)

Two different molecular cascades activate apoptosis (refer pages 24 to 26).

1. **Extrinsic (death receptor) pathway:** It is initiated when certain ligands (e.g. CD95L, TNF, FasL) bind to death receptor expressed on the surface of plasma membrane.
2. **Intrinsic (mitochondrial) pathway:** It is activated by a various stimuli (e.g. withdrawal of survival factors, stress and injury). This pathway is most commonly disabled in cancer.
 - Activation of intrinsic pathway → leads to **increased permeability of the mitochondrial outer membrane** → **releases cytochrome c and SMAC (second mitochondrial activator of caspases)** → initiate apoptosis.
 - Integrity of the mitochondrial outer membrane is controlled by: (1) **pro-apoptotic** and (2) **antiapoptotic proteins**.
 - **Pro-apoptotic proteins BAX and BAK** → increase mitochondrial permeability → initiate apoptosis.
 - **Antiapoptotic proteins BCL2, BCL-XL and MCL1:** Belong to BCL2 family of proteins, inhibit the action of proapoptotic proteins.
 - **Regulator of balance between proapoptotic and antiapoptotic proteins:** It is achieved by BH3-only proteins and includes BAD, BID and PUMA.

Methods of Evasion of Apoptosis and Associated Tumors

Tumor cells may escape or undergo reduced apoptosis. **Reduced apoptosis may be due to activation of either antiapoptotic proteins or reduced proapoptotic activity.**

1. **Activation of antiapoptotic BCL2:** For example, **follicular lymphomas** (about 85%) show a characteristic chromosomal translocation, t(14;18), causing **overexpression** of the **antiapoptotic BCL2 protein**. Neoplastic B lymphocytes are **protected from undergoing apoptosis** and survive for long periods.
2. **Reduced levels of proapoptotic BAX:** The p53 induces apoptosis of cells that are unable to repair DNA damage partly by transcriptional activation of **proapoptotic BAX**. **Mutation of p53 leads to reduced levels of BAX** resulting in decreased apoptosis.

Chemotherapeutic drugs can cause: Both necrosis and apoptosis.

BCL2: An antiapoptotic gene activated by t(14;18) translocation in majority of follicular B-cell lymphoma.

BCL2 gene family: Constitutes antiapoptotic genes.
BAX gene: Apoptotic gene.

Loss of Replicative Senescence

Q. Write briefly on telomerase activity.

All cancers contain immortal cells with unlimited capacity to replicate (cellular immortalization). Probably three interrelated factors appear to be involved in the immortality of cancer cells: (1) loss of senescence; (2) loss of mitotic crisis; (3) the capacity for self-renewal.

- **Loss of senescence:** Most **normal cells** have a limited capacity to undergo **cell division (replication) for about 60–70 times**. After this, the cells cannot divide (arrest of growth) and become senescent by permanently leaving the cell cycle and without any cell division. Cancer cells evade the process of senescence and retain the ability to reproduce. The senescence is probably associated with upregulation of tumor suppressors (e.g. p53 and INK4a/p16). These tumor suppressors maintain RB in a hypophosphorylated state that favors cell cycle arrest. RB-dependent G₁/S cell cycle checkpoint is disrupted in almost all cancers by a wide variety of acquired genetic and epigenetic aberrations.
- **Loss of mitotic crisis:** Cells resistant to senescence have increased capacity to replicate. However, these are not immortal and finally undergo **mitotic crisis** and die. This is due to progressive shortening of **telomeres**. **Telomeres (refer page 33) are the special structures present at the ends of chromosomes. During each cell division, a small section of the telomere is not duplicated resulting in progressive shortening, which is responsible for the limited replicative property of a cell. The shortening of telomere is prevented by an enzyme called telomerase.**
 - **Activation of telomerase:** Telomerase is expressed at very low levels in most somatic cells and with each cell division their telomeres shorten. Thus, any cells that escape from senescence die in mitotic crisis. However, if cells in crisis reactivate telomerase, these cells can restore their telomeres and survive. The cells damaged by oncogenes and tumor suppressor genes during crisis are at high risk for malignant transformation. Cancers may arise from stem cells which express telomerase. Whatever the mechanism, telomere is maintained in almost all types of cancers, and in 85–95% of cases it is due to upregulation of telomerase.
- **Self-renewal:** Tissue stem cells and germ cells express telomerase. Hence, they are resistant to mitotic crisis, and avoid the genetic and epigenetic alterations that trigger senescence. The long-lived stem cells have the capacity for self-renewal (refer Chapter 3), i.e. each time a stem cell divides at least one of the two daughter cells remains as a stem cell. Since cancers are immortal and have limitless proliferative capacity, they also may contain cells that can self-renew, and are called as *cancer*

stem cells. It is not clear whether cancer stem cells arise from the transformation of tissue stem cells or from the conversion of conventional somatic cells to transformed cells. In chronic myelogenous leukemia (CML), tumor cell subset with the *BCR-ABL* fusion gene has all the properties of a normal hematopoietic stem cell. Thus, CML appears to arise from a transformed hematopoietic stem cell.

Increased Angiogenesis

- Under homeostatic conditions, there is a balance between factors that favor new blood vessel formation (angiogenic factors/angiogenic promoters) and those hinder it (antiangiogenic factors/angiogenesis inhibitors).
- **Solid tumors** even though have all the genetic aberrations that are required for malignant transformation; their **growth requires increased supplies of nutrients and oxygen**. This in turn, requires proliferation of blood vessels (i.e. vascularization of tumors). In growing cancers **angiogenic factors promote angiogenesis** during which vessels sprout from previously existing capillaries (refer angiogenesis in Chapter 3). Thus, angiogenesis is an essential feature of malignancy. However, these vessels are not entirely normal. They are leaky and dilated and have a haphazard pattern of connection.

Effects of Neovascularization on Tumor Growth

- **Perfusion** supplies required nutrients and oxygen and remove waste products.
- Newly formed endothelial cells **secrete growth factors** [e.g. insulin-like growth factors (IGFs), platelet derived growth factors (PDGF)] which stimulate the growth of adjacent tumor cells.
- Permits access of tumor cells to these abnormal vessels and **contributes to metastasis**.

Mechanism of Angiogenesis

- During early phase of development, most tumors do not induce angiogenesis and tumors remain in a stage of vascular quiescence and starved of nutrients. During this phase, the tumor remains small or in situ, probably for years, till an **angiogenic switch** terminates this stage.
- **Molecular basis of the angiogenic switch**: This may be due to increased production of angiogenic factors and/or loss of angiogenic inhibitors. The source of these factors may be the tumor cells or by inflammatory cells (e.g. macrophages) or other stromal cells associated with the tumors.

Mediators of Tumor Angiogenesis

- **Family of VEGFs**: Relative lack of oxygen due to hypoxia triggers angiogenesis through the actions of HIF-1 α (an oxygen-sensitive transcription factor) on the transcription of the proangiogenic factor VEGF and bFGF. Gain-of-function mutations in RAS, MYC and MAPK signaling also upregulate VEGF expression and stimulate angiogenesis.
- **Mutations involving tumor suppressors and oncogenes**: In cancers, this tilts the balance in favor of angiogenesis. E.g. normal p53 stimulate the synthesis of the angiogenesis inhibitor thombospondin-1 and suppresses the expression of proangiogenic molecules such as VEGF. Mutation of these genes favor angiogenesis.
- **Angiopoietins**: Angiopoietin-2 is a family of vascular growth factor which favors formation of tumor blood vessel, stabilizes growing blood vessels and stimulates pericytes to surround the developing blood vessels.

Invasion and Metastasis

Refer page 175.

Evasion of Host Immune System

Normal immune system distinguishes self from non-self molecules and is very effective against infectious agents. Probably protective immunologic responses may be elicited against unique “tumor-specific antigens.”

Cancer cells can evade the host response. The term **immune surveillance** indicates that normal immune system constantly “scan” the body for malignant cells and destroy them. Tumors produce many factors that promote immune tolerance and immune suppression. Evasion of host immunity is a hallmark of many cancers.

Tumor Antigens

Antigens found in tumors that elicit an immune response have been found in some cancers. Tumor antigens can be classified according to their molecular structure and source.

1. **Products of mutated genes.** Neoplasms occur due to mutations in proto-oncogenes and tumor suppressor genes. These mutated genes produce various proteins which are recognized as nonself.
2. **Overexpressed or abnormally expressed cellular proteins:** Tumor antigens may also be normal cellular proteins that are abnormally expressed in tumor cells. The immune system can respond to this normal self-antigen.

3. **Antigens produced by oncogenic viruses:** Several viruses are associated with cancers. These viruses produce proteins that are recognized as foreign by the immune system. E.g. proteins produced by human papillomavirus (HPV) and Epstein-Barr virus (EBV). Cytotoxic T-cells (CTLs) recognize these antigens. A competent immune system is able to recognize and kill virus-infected cells.
4. **Oncofetal antigens** (refer page 211): They are proteins that are expressed at high levels on cancer cells and in normal developing (fetal) tissues. However, they are not limited to tumors and may be increased in tissues and blood in various inflammatory conditions, and found in small amount in normal tissues. They are not important targets of antitumor immunity. However, they can be used as markers that aid in the diagnosis of tumor and clinical management. E.g. carcinoembryonic antigen (CEA) and α -fetoprotein (AFP).
5. **Tumor cell surface glycolipids and glycoproteins:** Most human tumors express higher than normal levels and/or abnormal forms of surface glycoproteins and glycolipids. They may be of diagnostic value and target for therapy. These include gangliosides, blood group antigens and mucins (e.g. CA-125 and CA-19-9, expressed on ovarian carcinomas, and MUC-1 expressed on both ovarian and breast carcinomas).
6. **Differentiation antigens:** These molecules seen in normal cells (normal self-antigens) of the same origin as cancer cells. They do not induce immune responses in tumor-bearing hosts. E.g. CD20, which is a normal B-cell differentiation antigen, is expressed by some lymphomas, and anti-CD20 antibody (rituximab) is used for the treatment mature B-cell lymphomas and leukemias.

Antitumor Mechanisms

Cell-mediated immunity is the major antitumor mechanism. Although cancer patient's sera may contain antibodies that recognize tumors, they do not have protective role.

- **Cytotoxic T lymphocytes** (CD8⁺ CTLs): They react against tumor antigens. They have protective role against virus-associated neoplasms (e.g. EBV- and HPV-induced tumors), and associated with better prognosis in several cancers.
- **Natural killer (NK) cells:** They can kill tumor cells without prior sensitization and thus may be the first line of defense against tumor cells.
- **Macrophages:** Activated macrophages may kill tumors by mechanisms similar to those used to kill microbes (e.g. production of reactive oxygen species).

Escape of Immune Surveillance

Immunosurveillance is a process by which immune system recognizes transformed cells and destroys tumor cells in order to inhibit the growth of tumor tissue. Increased frequency of cancers is observed in patients with immunodeficiency (e.g. congenital immunodeficiencies, immunosuppressed transplant recipients and persons with AIDS). However, most cancers develop in patients without any overt immunodeficiency. So in an immunocompetent host, tumor cells must develop mechanisms to escape or evade the immune system and immune surveillance. These mechanism include:

- **Elimination of strongly immunogenic subclones and selective outgrowth of antigen-negative variants.**
- **Loss or reduced expression of MHC molecules by tumor cells.**
- **Activation and engagement of immunoregulatory pathways** that serve as "checkpoints" in immune responses, thereby inhibiting tumor immunity.
- **Secretion of immunosuppressive factors by cancer cells** which inhibit the host immune response. E.g. TGF- β is secreted in large quantities by many tumors is a potent immunosuppressant.
- **Induction of immunosuppressive regulatory T-cells (Tregs).**

GENOMIC INSTABILITY

Q. Write briefly on genomic instability.

We swim in environmental agents that are mutagenic (e.g. chemicals, radiation, sunlight). Thus, DNA is under relentless assault from many environmental agents (exogenous stresses) as well as internal stresses such as reactive oxygen species (ROS), etc. that can damage cellular DNA. However, cancers are relatively rare outcomes of these encounters. Reasons for this is that the cells maintain genomic stability through different mechanisms that detect and repair DNA damage, cause the death of cells with irreparable damage, oncogene-induced senescence and immune surveillance. As discussed earlier, *TP53* tumor suppressor gene protects the genome from oncogenic damage, 1) by arresting cell division to provide time for repair of DNA damage caused by environmental mutagens and 2) by initiating apoptosis in irreparably damaged cells. Genes which repair DNA are called as **DNA repair genes which protect the integrity of the genome.**

- Normally, DNA repair genes repair nonlethal damage in other genes including proto-oncogenes, tumor suppressor genes and genes that regulate apoptosis. Mutations of these DNA repair genes do not directly

transform cells. Loss-of-function mutations (disability) involving DNA repair genes contribute to carcinogenesis (neoplastic transformation) indirectly by impairing the ability of the cell to recognize and repair nonlethal genetic damage in other genes. These affected cells acquire mutations at an accelerated rate, a state referred to as a **mutator phenotype** and it is marked by **genomic instability**.

- Genomic instability may be due to either **microsatellite instability** (single or oligo-nucleotide mutations) or more commonly due to **chromosomal instability** leading to aneuploidy (abnormal number of chromosomes in a cell).

Types of DNA Repair Genes (Flowchart 7.1)

1. **Mismatch repair:** After DNA replication is complete; **mismatch repair genes act as spell checkers or proofreaders, and excise and replace the mismatched nucleotides.** Defect in these genes → mismatched nucleotide errors gradually accumulate in the genome. These errors may involve **proto-oncogenes and tumor suppressor genes**.

Hereditary nonpolyposis colon cancer syndrome: HNPCC syndrome (Lynch syndrome) is characterized by **familial predisposition to the development of carcinomas of the colon** affecting predominantly the cecum and proximal colon. **It is due to defects in DNA mismatch repair gene.**

- **Microsatellite instability:** One of the characteristics of patients with mismatch-repair defects is microsatellite instability. **Microsatellites are tandem repeats of one to six nucleotides found throughout the genome.** Normally the length of these microsatellites remains constant. In individuals with HNPCC, **these satellites are unstable and increase or decrease in length** in tumor cells, creating alleles not found in normal cells of the same patient.

- Each affected patient inherits one defective copy of a DNA mismatch-repair gene and acquires the second hit of DNA mismatch-repair gene in colonic epithelial cells. Thus, **mode of inheritance of DNA-repair genes is like tumor suppressor genes.**
2. **Nucleotide excision repair: Example—xeroderma pigmentosum.**
 - It is an **inherited disorder** of defective **nucleotide excision repair gene**.
 - These patients have an **increased risk** for the development of **skin cancers following exposure to the UV light** present in sun rays.
 - **UV radiation causes cross-linking of pyrimidine residues, preventing normal DNA replication.** Such DNA damage is normally **repaired by the nucleotide excision repair system**.
 3. **Recombination repair:** Recombination is a process in which random crossing over of double-stranded DNA occurs between two parental homologous chromosomes. This occurs by breakage of homologous DNA molecules and rejoining of the parts in new combinations. It is a necessary process in meiosis and involves exchange of genetic information. Recombination also occur during mitosis at a predictable rate. Exposure to ionizing radiation significantly increases the rate of breakage in chromosomes. Usually, these breakages are accurately repaired by recombination repair genes. Disorders associated with recombination repair genes include Bloom syndrome, ataxia-telangiectasia, and Fanconi anemia. *BRCA1* and *BRCA2* are mutated in familial breast cancer and both are associated with many proteins involved in the homologous recombination repair pathway.

DNA repair genes: Enzymes causing excision of dimers include endonuclease, exonuclease and polymerase ligase.

DNA repair genes: Inherited mutations are associated with increased risk of cancer.

Xeroderma pigmentosum: Defect in the nucleotide excision repair gene → increased risk for cancer of skin exposed to UV light.

Microsatellites: Tandem repeats of one to six nucleotides found in the genome.

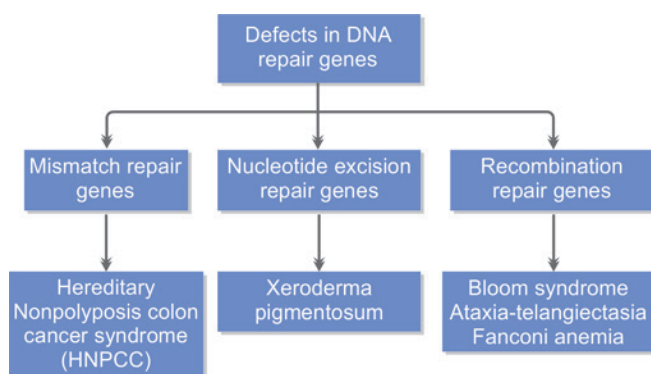
DNA contains several repeat sequences of three nucleotides (trinucleotide). If repeat sequences are directly adjacent to each other they are called as tandem repeats.

Syndromes associated with defects in recombination repair gene:

1. Bloom syndrome
2. Ataxia-telangiectasia
3. Fanconi anemia.

They have hypersensitivity to DNA damaging agents (e.g. ionizing radiation).

Flowchart 7.1: Different types of DNA repair gene defects and associated conditions



ETIOLOGY OF CANCER (CARCINOGENIC AGENTS)

Q. Classify carcinogens /enumerate the types of carcinogens.

Definition: A **carcinogen** is an **agent known or suspected to cause tumors** and such agents are said to be **carcinogenic** (cancer causing).

Carcinogenic agents (Fig 7.23): (1) chemicals, (2) microbial agents, and (3) radiation.

Chemical Carcinogenesis

Q. List major chemical carcinogens and describe in detail chemical carcinogenesis.

Sir Percival Pott (London surgeon) first related **scrotum skin cancer in chimney sweeps to a specific chronic chemical exposure to soot**. Based on this, a rule was made that chimney sweep members must bathe daily and this public health measure controlled scrotal skin cancer. Japanese investigators (Yamagiva and Ichikawa) experimentally produced skin cancers in rabbits by using coal tar. Subsequently, hundreds of chemical carcinogens were discovered.

Classification of Chemical Carcinogens

Chemical carcinogens may be classified into two categories: Direct acting and indirect acting. Major chemical carcinogens are listed in Box 7.1.

Direct-acting Agents

Direct-acting chemical agents **do not require metabolic conversion** to become carcinogenic, but most of them are **weak carcinogens**. Some of the drugs (e.g. alkylating agents) used to cure, control, or delay recurrence of some cancer (e.g. leukemia, lymphoma), may produce a second form of cancer (e.g. acute myeloid leukemia) later.

• Alkylating agents:

- **Source:** Many cancer chemotherapeutic drugs (e.g. cyclophosphamide, cisplatin, busulfan) are alkylating agents.

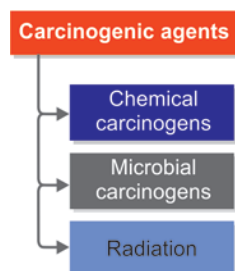


Fig. 7.23: Major types of carcinogenic agents

BOX 7.1: Major chemical carcinogens

DIRECT-ACTING CARCINOGENS

1. Alkylating Agents

- β -Propiolactone
- Anticancer drugs (cyclophosphamide, chlorambucil, nitrosoureas, etc.)
- Dimethyl sulfate
- Diepoxbutane

2. Acylating Agents

- 1-Acetylimidazole
- Dimethyl carbamyl chloride

INDIRECT-ACTING CARCINOGENS (PROCARCINOGENS)

1. Polycyclic and Heterocyclic Aromatic Hydrocarbons

- Benz[a]anthracene
- Benzo[a]pyrene
- Dibenzo[a,h]anthracene
- 7,12-Dimethylbenz[a]anthracene
- 3-Methylcholanthrene

2. Aromatic Amines, Amides and Azo Dyes

- 2-Naphthylamine (β -naphthylamine)
- Benzidine
- 2-Acetylaminofluorene
- Dimethylaminoazobenzene (butter yellow)

NATURAL PLANT AND MICROBIAL PRODUCTS

- Aflatoxin B₁
- Griseofulvin
- Betel nuts

OTHERS

- Nitrosamine and amides
- Vinyl chloride
- Metals : Nickel, chromium
- Insecticides, fungicides
- Asbestos

- **Mechanism of action:** Alkylating agents contain **electron-deficient atoms that react with electron-rich atoms in DNA**. These drugs not only destroy cancer cells by damaging DNA, but also injure normal cells.
- **Cancers produced:** Solid and hematological malignancies.

Direct-acting chemical agents: Do not require metabolic conversion to become carcinogenic, but are weak carcinogens.

Alkylating agents: Solid and hematological malignancies.

Indirect-acting Agents (Procarcinogens)

Q. Write short note on polycyclic hydrocarbons.

These chemicals **require metabolic activation** for conversion to an active ultimate carcinogen.

1. **Polycyclic aromatic hydrocarbons:** They are the **most potent** and extensively studied indirect-acting chemical carcinogens. Examples: Benzo(a) pyrene, 3-methylcholanthrene, and dibenzanthracene.

– **Source:**

- ◆ Originally derived from coal tar and fossil fuels.
- ◆ Cigarette smoke: Polycyclic aromatic hydrocarbons are formed during high-temperature combustion of tobacco in cigarette smoking → responsible for lung cancer in cigarette smokers.
- ◆ Animal fats: It may produce it during the process of broiling meats.
- ◆ Smoked food: Examples, smoked meats and fish.

– **Mechanism of action:**

- ◆ Polycyclic hydrocarbons are metabolized by cytochrome P450-dependent mixed function oxidases to electrophilic (have electron-deficient atoms) epoxides.
- ◆ Epoxides react with proteins and nucleic acids (DNA, RNA). Example: Polyvinyl chloride (used in plastic industry) is metabolized to an epoxide → causes hepatic angiosarcomas.

- **Cancers produced:** The specific type of cancer produced depends on the route of administration. Examples: Cancers in the **skin, soft tissues, lung and breast.**

Polycyclic hydrocarbons: Lung cancer.

Workers exposed to polyvinyl chloride may develop angiosarcoma of liver.

Indirect-acting carcinogen needs metabolic activation for their conversion into DNA-damaging agent.

2. **Aromatic amines and azo dyes:** They are indirect-acting carcinogens.

– **Source:**

- ◆ In the past, the aromatic amines (β -naphthylamine) and azo dyes were used in the aniline dye and rubber industries.
- ◆ Azo-dyes were used for coloring food (e.g. butter and margarine, which give yellow color, scarlet red for coloring cherries).

– **Mechanism of action:**

- ◆ They are not carcinogenic at the point of application.
- ◆ Both aromatic amines and azo dyes are mainly metabolized in the liver.
- ◆ The aromatic amines are converted to active carcinogens in the liver. However, can be detoxified

immediately by conjugation with glucuronic acid in the liver.

- ◆ The conjugated metabolite is excreted in the urine and deconjugated in the urinary tract by the enzyme glucuronidase. The urothelium is thus exposed to the active carcinogen (reactive hydroxylamine) which may cause bladder cancer.

- **Cancers produced: Bladder cancer** (β -naphthylamine and benzidine) and **liver tumors** (azo dyes).

Aromatic amines: Bladder and liver cancers.

Natural Microbial Product

- Aflatoxin B₁

- **Source:** Aflatoxin B₁ is a natural product of *Aspergillus flavus*, a mold which grows on **improperly stored grains and peanuts.**

- **Mechanism of action:** Metabolized to an **epoxide** and bind to DNA and also produces **mutations of p53 gene.**

- **Cancers produced :** Powerful liver carcinogen → **hepatocellular carcinoma.**

Aflatoxin: Hepatocellular carcinoma.

Others

- **Nitrosamines:** They are potent carcinogens.

- **Source:** **Before the advent of refrigerator**, nitrites were added as a **preservative for meats and other foods.**

- **Mechanism of action:** **Nitrites react with amines and amides** in the diet and are metabolized by commensal bacteria within the gut and converted to **carcinogenic nitrosamines.**

- **Cancers produced:** Mainly **gastrointestinal neoplasms.**

- **Metals:** Compounds like arsenic, nickel, lead, cadmium, cobalt, chromium and beryllium can produce cancer. Most metal-induced cancers occur due to occupational exposure.

- **Asbestos:** Inhalation of asbestos fibers → results in asbestosis, pleural plaques, **mesothelioma** and **carcinoma of the lung.** Mesothelioma may involve pleura as well as peritoneum.

Detection of carcinogenicity of a chemical: Mutagenicity testing of chemical is done by **Ames test.** The appearance of frameshift mutations and base-pair substitutions in a culture of **bacteria of a Salmonella species** indicates that the chemical tested is carcinogenic.

Mechanism of Action of Chemical Carcinogens

Molecular targets of chemical carcinogens: Most chemical carcinogens are mutagenic. A **mutagen** is an agent, which can permanently alter the genetic constitution of a cell.

- All direct and ultimate carcinogens (of indirect carcinogens) contain **highly reactive electrophilic groups** → form adducts with DNA, RNA and proteins.
- **Genes affected:** Any gene may be affected but commonly involved are **proto-oncogenes (RAS)** and **tumor suppressor genes (p53)**.

Multistep Hypothesis (Fig. 7.24)

Q. Multistep carcinogenesis.

Chemical carcinogenesis is a **multistep** process. Once the tumor process is started, it does not require the continued presence of the carcinogen.

Four steps involved in chemical carcinogenesis are:

1. **Initiation:** It is the **first important step** that develops from **exposure of cells** to a sufficient dose of a **carcinogenic agent** (initiator).
 - **Reaction with DNA:** All initiators are highly reactive electrophiles (electron-deficient atoms) and can react with nucleophilic (electron-rich) sites in the cell. Sites of reaction of initiation are DNA, RNA and proteins.
 - **Effect of initiation:** Initiators produces **nonlethal permanent (irreversible) alterations or damage to DNA (mutations)** in a cell. If damage is lethal or severe it causes cell death.

Q. Promoters in carcinogenesis.

Q. Differences between initiators and promoters.

2. **Promotion**
 - **Promoters:** They are **noncarcinogenic** agents and **cannot directly damage DNA** (mutation).
 - **Cell proliferation:** Promoters stimulate the initiated (with permanent DNA damage- mutated) cells

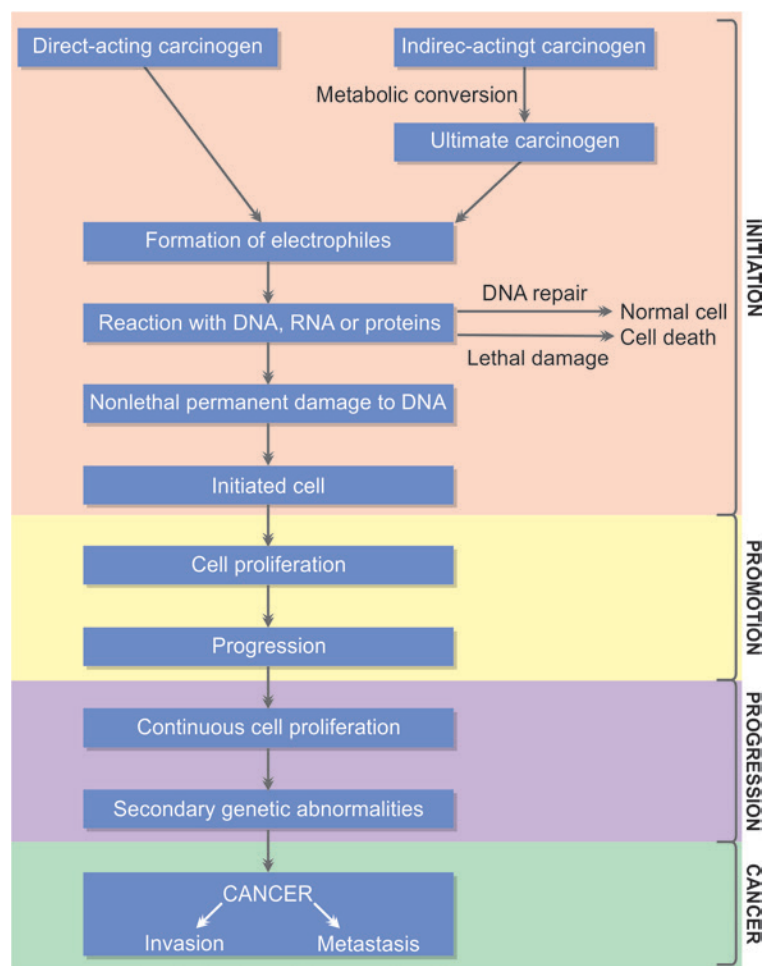


Fig. 7.24: Multistep theory of chemical carcinogenesis

Initiators: Cause irreversible damage to DNA.

Promoters: Cause reversible damage to DNA.

Ames test: To detect carcinogenicity of a chemical.

Mutagen: Agent that can permanently alter the genetic constitution of a cell.

Most chemical carcinogens are mutagenic.

Multistep theory of chemical carcinogenesis:

1. Initiation
2. Promotion
3. Progression
4. Cancer.

to enter into the cell cycle → cell proliferation. Unlike initiators, the cellular changes produced by promoters are reversible.

- **Produce changes only on initiated cell:** Tumors develop only if the promoter is applied after the initiator and not the reverse way.
- **Examples of promoters include:** phorbol esters, hormones, phenols and drugs.

Promoters: Noncarcinogenic agents and cannot directly damage DNA (mutation). After exposure of a cell to initiator, promoters stimulate these initiated cells.

3. **Progression:** Continuous proliferation of initiated cells → leads to secondary genetic abnormalities → tumor growth becomes independent of the initiator or the promoter (i.e. autonomous). Many accumulated mutations finally immortalize the cells.
4. **Cancer:** Final result of the different steps is the development of neoplasm → invasion → metastases.

Examples: The morphologic sequence of hyperplasia, dysplasia and carcinoma in situ found in epithelium (e.g. skin, cervix and colon) indicate multistep carcinogenesis.

Microbial Carcinogenesis

Q. Classify/List oncogenic viruses.

Viruses that cause tumors are called as oncogenic viruses. Many viruses have been proved to be oncogenic in animals, but only a few have been associated with human cancer.

Microbial carcinogens: Viruses > bacteria > parasites

Classification (Fig. 7.25): They are mainly classified depending on the genetic material into: (1) oncogenic RNA viruses and (2) oncogenic DNA viruses.

Oncogenic RNA Viruses

Q. Discuss the role of RNA viruses in tumorigenesis.

Q. Explain the mechanism involved in tumor production by viruses.

Human T-cell leukemia virus type 1: It is a retrovirus.

- **Major target** for neoplastic transformation: CD4⁺ T lymphocytes.
- **Tumor caused:** Adult T-cell leukemia/lymphoma—develops after a long latent period (20–50 years).
- **Mode of infection:** (1) Sexual intercourse, (2) blood products and (3) breast feeding.
- **Mechanism of oncogenesis (Fig. 7.26):** It is a multistep process.
 - HTLV-1 infects CD4⁺ T-cells. HTLV-1 does not contain oncogene and its genes cannot integrate into the host genome.
 - HTLV-I contains **TAX gene** and actions of its product TAX protein are:
 - ◆ Required for viral replication and cellular transformation.
 - ◆ Activates other genes involved in T-cell proliferation and differentiation. These include genes that code for:
 1. Interleukin (IL)-2 and its receptor (IL-2R)
 2. IL-15 and its receptor IL-15R
 3. Granulocyte macrophage colony-stimulating factor (GM-CSF).
 - **Inactivates: p53 and other genes controlling cell cycle** (e.g. *CDKN2A/p16* gene).
- Secretion of cytokines and autocrine stimulation of CD4⁺ T-cells → proliferation of **nonmalignant polyclonal cells**.
- Tax protein also stimulates secretion of GM-CSF by CD4⁺ T-cells → stimulates nearby macrophages to produce

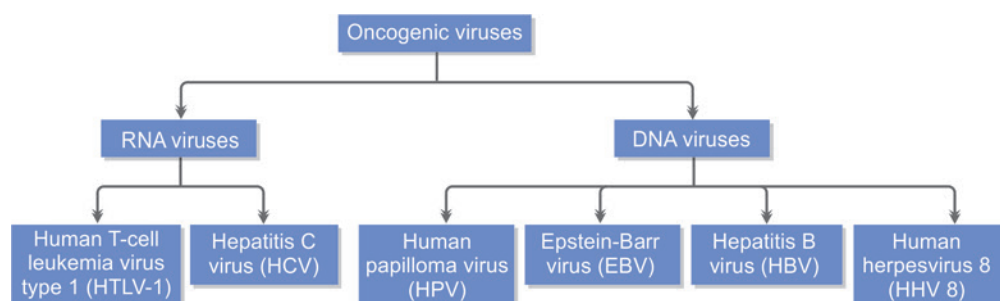


Fig. 7.25: Classification and types of oncogenic viruses

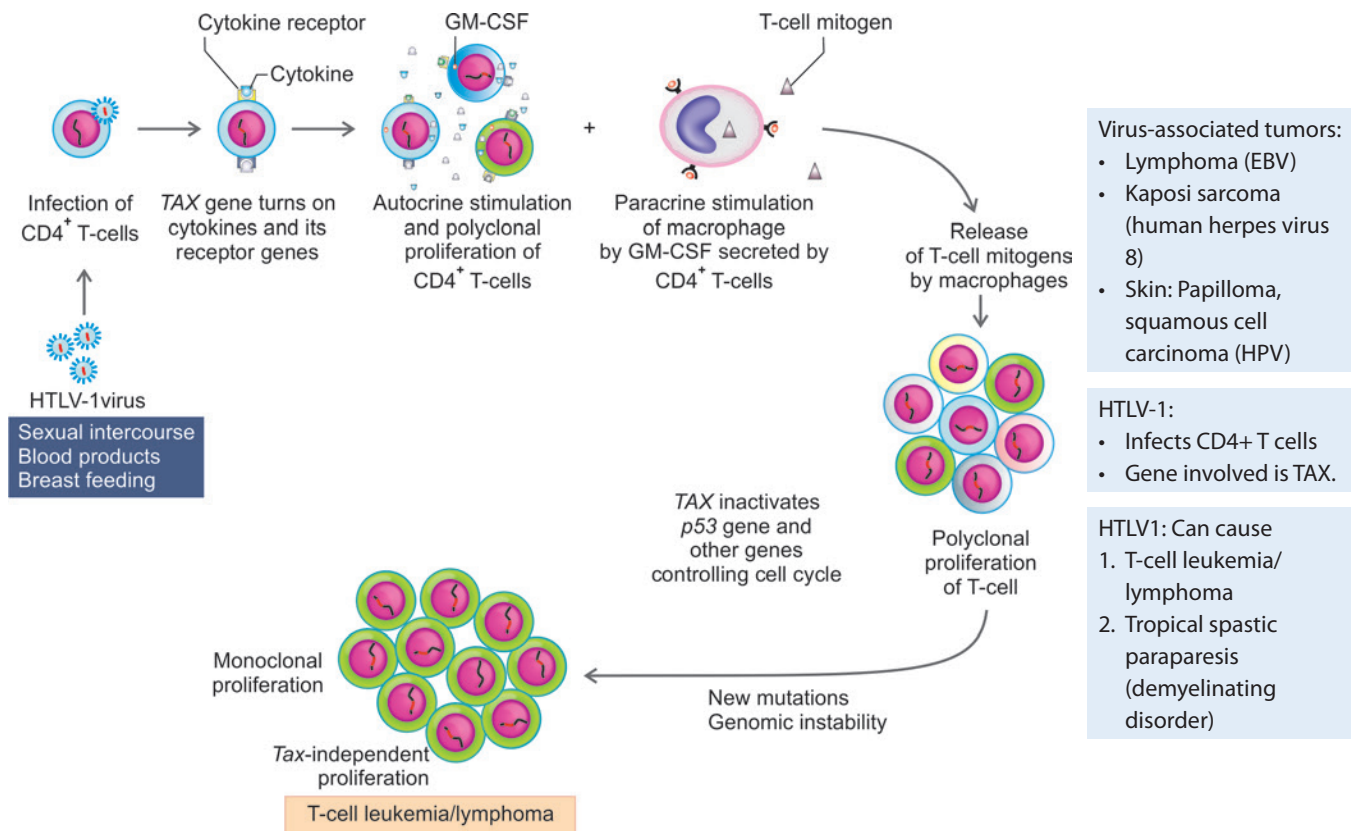


Fig. 7.26: Pathogenesis of human T-cell leukemia

T-cell mitogens → polyclonal proliferation of CD⁺⁺ T-cells.

- TAX inactivates *p53* and other genes controlling cell cycle → **increased risk of developing mutations and genomic instability** in proliferating CD4⁺ T-cells.
- Accumulation of mutations and chromosomal abnormalities → **monoclonal neoplastic proliferation of CD4⁺ T-cell**.

Oncogenic DNA Viruses

Oncogenic DNA viruses:

1. Human papillomavirus (HPV)
2. Epstein-Barr virus (EBV)
3. Hepatitis B virus (HBV)
4. Kaposi sarcoma herpes virus (KSHV), also called human herpes virus 8 (HHV-8)
5. Merkel cell polyomavirus causing Merkel cell carcinomas.

Five DNA viruses can cause cancer. HCV is not a DNA virus and found to be associated with cancer.

Human Papillomavirus (HPV)

Q. Write short note on oncogenesis by human papillomavirus.

- **Cell infected:** Human papillomaviruses (HPV) infects **only the immature squamous cells** but its **replication occurs in the maturing, nonproliferating squamous cells**. Thus, their full productive life cycle occurs only in squamous cells. The physical state of the virus differs in different lesions.
- **Types of HPV and associated lesions** (Table 7.13): More than 70 genetically different types of HPV have been identified. They are divided into **low-risk and high-risk HPVs**.

Mode of action (Fig. 7.27)

Episomal form: In **benign lesions** such as benign warts, condylomata and most **precancerous lesions**; the HPV genome is present as nonintegrated, free (episomal) viral DNA.

Integration: In **cancers**, the HPV genome is integrated into the host genome and is essential for malignant

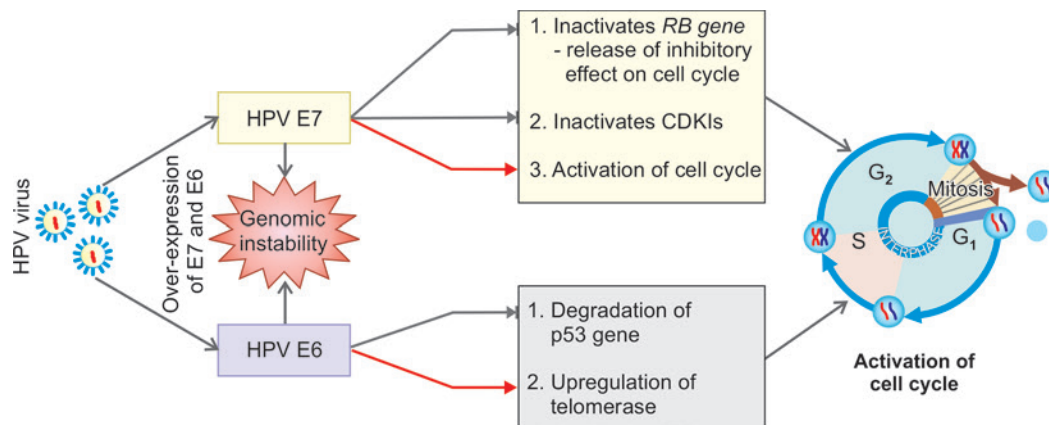


Fig. 7.27: Mode of action of HPV proteins E6 and E7 on the cell cycle

transformation. **Integration results in overexpression of the two viral genes E6 and E7.** Protein products of E6 and E7 (oncoproteins) are important for the oncogenic effects of HPV.

- **Actions of E7 protein:**
 - **Inactivation of tumor suppressor RB gene:** E7 protein binds to the hypophosphorylated (active) form of RB protein → releases its inhibitory effect on cell cycle progression (Fig. 7.27).
 - **Inactivation of inhibitors of cell cycle:** For example, inactivation of CDKIs (CDKN1A/p21 and CDKN1B/p27) → activates cell cycle.
 - **Activation of cyclins (activators of cell cycle):** These include cyclins E and A → facilitates G₂/M transition → activation of cell cycle.
- **Actions of E6 protein:** The E6 protein complements the effects of E7.
 - **Inactivation of tumor suppressor p53 gene:** E6 binds and degrades p53 → degrades BAX (a proapoptotic factor) → prevents apoptosis.
 - **Activation of telomerase:** E6 stimulates the expression of TERT (the catalytic subunit of telomerase → prevents replicative senescence and cell proliferation continues.
- **Combined action of E6 and E7:** They induce centrosome duplication and genomic instability.

High-risk HPV types express E6 and E7 that causes:

- Inactivation of tumor suppressor genes—RB and p53
- Activation of cyclins
- Inhibition of apoptosis
- Activation of telomerase.

HPV: Genes involved—

1. E6
2. E7.

Epstein-Barr Virus (EBV)

Q. Write short note on Epstein-Barr virus, diseases caused and cancers.

EBV is a human herpesvirus, which infects B lymphocytes. Patients may manifest as a short-lived infectious mononucleosis or develop few human cancers. The list of cancers produced include:

1. African form of Burkitt lymphoma.
2. B-cell lymphomas in immunosuppressed (e.g. HIV infection or immunosuppressive therapy after organ transplantation).
3. A subset of Hodgkin lymphoma.
4. Nasopharyngeal carcinoma (T-cell tumor).
5. Some gastric carcinomas.
6. Rare forms of T-cell lymphomas and natural killer (NK) cell lymphomas.
7. Very rarely sarcomas.

EBV: African form of Burkitt lymphoma

Pathogenesis (Fig. 7.28): EB virus infects B lymphocytes by binding to the membrane receptor CD21 (CR2). The infection of B-cells may be either productive (lytic) or latent.

- **Productive/lytic infection:** It develops only in a few patients and results in death of infected cells → release of virions → infection of other B-cells.
- **Latent infection:** It occurs in majority of the cases. The virus becomes latent inside the B-cells → are transformed or “immortalized” so that they are capable of proliferation indefinitely. **Immortalization of B lymphocyte is the hallmark of EBV infection.** Molecular basis of B-cell immortalization is related to two EBV-coded genes and viral cytokines.

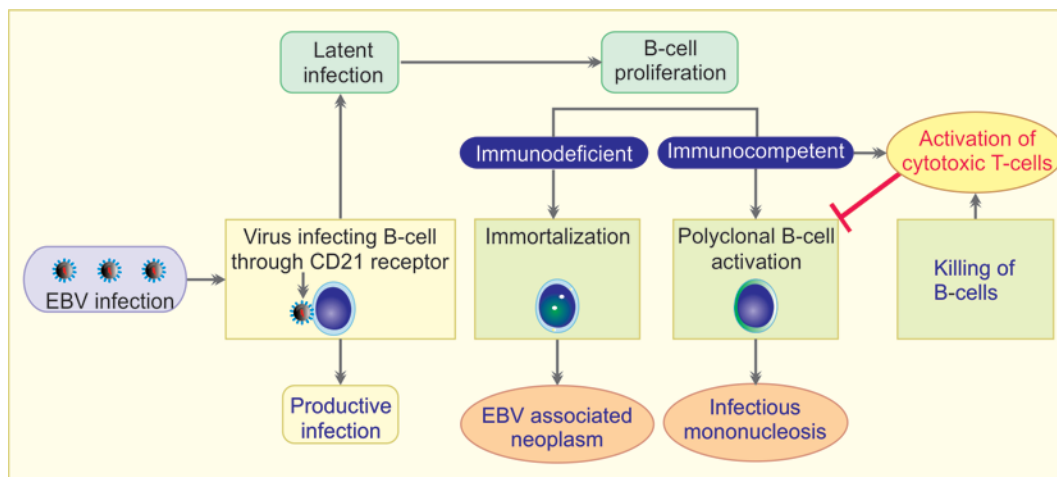


Fig. 7.28: Pathogenesis of EBV infection

1. **LMP1** (latent membrane protein 1): It acts as an **oncogene** → activates the NF-κB and JAK/STAT signaling pathways → **promote B-cell survival** (prevents apoptosis by activating Bcl-2) and **proliferation**.
2. **EBNA2** (Epstein-Barr nuclear antigens 2): It **stimulates transcription of many host genes**, including genes that drive the cell cycle (e.g. cyclin D) and the *SRC* family of proto-oncogenes.
3. **Viral cytokine (vIL-10)**: It is pirated from host genome, **prevent macrophages and monocytes from activating T-cells and killing viral infected cells**.

EBV: Genes involved—

1. LMP1
2. EBNA2
3. VIL-10
4. c-MYC in Burkitt lymphoma.

EBV-related oncogenesis: Evasion of immune system is the key step.

LMP-1 gene plays a role in oncogenesis induced by: Epstein-Barr virus.

African form of Burkitt lymphoma: It is a **B-cell neoplasm** and is the **most common childhood tumor in central Africa and New Guinea**. A morphologically similar lymphoma occurs sporadically throughout the world.

Burkitt lymphoma: EBV is not directly oncogenic, but acts as a polyclonal B-cell mitogen → favors **t(8;14) translocation** → activate the c-MYC oncogene → release the cells from normal growth regulation.

- **Mechanism of endemic Burkitt lymphoma:** Normally, EBV infects B-cells and stimulate B lymphocyte proliferation which is controlled by suppressor T-cells. Sequence of events in the pathogenesis of endemic African Burkitt lymphoma are:

- Accompanying infections (such as malaria or other infections) impairs immune competence → **lack of an adequate suppressor T-cell response** → allows **uncontrolled proliferation of B-cell**.
- EBV-infected B-cells expressing LMP-1 are **eliminated by immune system**.
- Lymphoma develops only when there are **chromosomal translocations** that activate the *c-MYC* oncogene (Fig. 7.18) → results in **uncontrolled proliferation** of a malignant clone of B lymphocytes.
- **Mechanism of nonendemic Burkitt lymphoma:** All tumors possess the t(8;14) or other translocations that dysregulate c-MYC.

Hepatitis B and C Viruses

Hepatitis C virus: Oncogenic RNA virus.

HBV is a DNA virus whereas **HCV is RNA virus**. There is a strong association between chronic infection with HBV and HCV (chronic hepatitis and cirrhosis) with primary **hepatocellular carcinoma**.

Mechanism: The oncogenic effects of both HBV and HCV are **multifactorial**.

- **Immunologically mediated chronic inflammation:** It causes **death of the hepatocytes**.
- **Compensatory liver cell regeneration:** It is aided by a several growth factors and cytokines produced by **activated immune cells of inflammation**.

- **Genomic damage and mutation:** It is due to mediators (e.g. reactive oxygen species) produced by activated immune cells.

HBV: HBV genome contains a viral regulatory gene known as **HBx**. Various actions of **HBx** are:

- Direct or indirect **activation of many transcription factors and signal transduction pathways.**
- **Inactivation of *p53***
- **HBV DNA can be integrated within the human genome and can cause multiple deletions,** which may harbor unknown tumor suppressor genes.

HCV: HCV genome, such as the **HCV core protein**, may **activate many growth-promoting signal transduction pathways** and cause tumor.

Human Herpesvirus 8 (HHV 8)

It is a DNA virus, which **infects the spindle cells of Kaposi sarcoma** and also **lymphocytes**.

Neoplasm produced:

- **Kaposi sarcoma:** It is a **vascular neoplasm**, which is the **most common neoplasm, associated with AIDS**. HHV 8 has also been found in Kaposi sarcoma from HIV-negative patients.
- **B-cell lymphoid malignancies:** Two uncommon lymphoid malignancies, namely **primary effusion lymphoma** and **multicentric Castleman disease** are associated with HHV 8.

Mechanism

- **HHV 8 viral genome encodes proteins, which interfere with the *p53* and *RB* tumor suppressor pathways.**
- **HHV 8 also encodes gene products, which downregulate class I major histocompatibility complex (MHC) expression** → infected cells **escape recognition by cytotoxic T lymphocytes**.

Various viruses implicated in human tumors are listed in Table 7.13.

HHV8:

1. Kaposi sarcoma
2. Primary effusion lymphoma
3. Multicentric Castleman disease

Kaposi sarcoma:

1. Vascular neoplasm
2. Most common neoplasm, associated with AIDS.

Bacteria

Helicobacter Pylori

Diseases caused by *H. pylori* are: (1) **peptic ulcers**, (2) **gastric adenocarcinomas** and (3) **gastric lymphomas**.

TABLE 7.13: Various viruses implicated in human tumors and associated lesions.

Type of virus	Lesions
ONCOGENIC RNA VIRUSES	
• Human T-cell lymphotropic virus type-1	Adult T-cell leukemia/lymphoma
• Hepatitis C Virus	Hepatocellular carcinoma
ONCOGENIC DNA VIRUSES	
1. Human papillomavirus	
A. Low-oncogenic risk HPV—benign lesions of squamous epithelium	
♦ HPV types 1, 2, 4 and 7	Benign squamous papilloma (wart)
♦ HPV-6 and HPV-11	Condylomata acuminata (genital warts) of the vulva, penis and perianal region Laryngeal papillomas
B. High-oncogenic risk HPV—malignant tumors	
♦ HPV types 16 and 18	Squamous cell carcinoma of the cervix and anogenital region Oropharyngeal cancers (tonsil)
2. Epstein-Barr virus	Burkitt lymphoma (requires cofactor-malaria) Nasopharyngeal cancer
3. Hepatitis B virus	Hepatocellular carcinoma
4. Human Herpes virus-8	Kaposi's sarcoma Pleural effusion lymphoma, multicentric Castleman disease
5. Merkel cell polyomavirus	Merkel cell carcinoma

1. Gastric adenocarcinomas

Mechanism: It is similar to that of HBV and HCV-induced hepatocellular carcinoma.

- **Chronic inflammation:** *H. pylori* causes chronic inflammation (chronic gastritis) → followed by gastric atrophy → intestinal metaplasia → dysplasia → cancer.
- **Genes:** *H. pylori* causing gastric adenocarcinoma contains **cytotoxin-associated A (*CagA*)** gene can penetrate into gastric epithelial cells → initiation of signals → unregulated growth factor stimulation.

2. Gastric lymphoma: *H. pylori* produces lymphoma of B-cell origin and are called as lymphomas of mucosa-associated lymphoid tissue, or **MALTomas**.

Neoplasms due to *Helicobacter pylori*:

1. Gastric adenocarcinoma
2. MALT lymphoma.

Helicobacter pylori: Gene involved is *CagA*

Fungi

Aspergillus flavus produces aflatoxin B: Hepatocellular carcinoma.

Fungi may cause cancer by producing toxic substances (mycotoxins). **Aflatoxin B₁** produced by *Aspergillus flavus* is a potent carcinogen responsible for **hepatocellular carcinoma**.

Parasites

Two parasites which can cause tumor are:

- ***Schistosoma*** is strongly implicated in **carcinoma of urinary bladder (usually of squamous cell type)**. The ova of the parasite can be found in the affected tissue.
- ***Clonorchis sinensis*** (Chinese liver fluke) lodges in the bile ducts → produces an inflammatory reaction, epithelial hyperplasia and sometimes **adenocarcinoma of the bile ducts (cholangiocarcinoma)**.

Clonorchis sinensis: Cholangiocarcinoma.

Schistosoma: Squamous cell carcinoma of urinary bladder.

Hormones

Hormones in the body may act as cofactors in carcinogenesis.

Estrogen

- **Endometrial carcinoma**: It may develop in females with estrogen-secreting **granulosa cell tumor of ovary** or those receiving exogenous estrogen.
- **Adenocarcinoma of vagina**: Increased frequency of adenocarcinoma of vagina is observed in **daughters of mothers who received estrogen during pregnancy**.
- **Abnormal vascularity of tumor**: Estrogens can make existing tumors abnormally vascular (e.g. adenomas and focal nodular hyperplasia).

Estrogen: Endometrial adenocarcinoma.

Androgenic and anabolic steroids: They may cause hepatocellular tumors.

Hormone-dependent Tumors

- **Prostatic carcinoma** usually responds to administration of estrogens or castration.
- **Breast carcinomas** regress following oophorectomy.

Radiation Carcinogenesis

Q. Write short note on radiation induced cancers.

Radiation is a well-known carcinogen.

Latency: Extremely long latent period is common and it has a cumulative effect. Radiation has also additive or synergistic effects with other potential carcinogenic agents.

UV rays cause skin cancer:

1. Squamous cell carcinoma
2. Basal cell carcinoma
3. Malignant melanoma.

Types of radiation: They are divided into two types, namely (1) ultraviolet (UV) rays of sunlight and (2) ionizing electromagnetic and particulate radiation.

Ultraviolet Rays

Lymphoid tissue: Most sensitive to radiation.

They are derived from the sunlight.

Tumors caused: Skin cancer, namely (1) **squamous cell carcinoma**, (2) **basal cell carcinoma** and (3) **malignant melanoma**. They are more common on parts of the body regularly exposed to sunlight and ultraviolet light (UVL).

Bone: Least sensitive to radiation.

Risk Factors

The amount of damage incurred depends on:

- **Type of UV rays**
- **Intensity of exposure**
- **Protective mantle of melanin**
 - **Melanin** absorbs UV radiation and has a protective effect.
 - Skin cancers are more common in fair-skinned people and those living in geographic location receiving a greater amount of sunlight (e.g. Queensland, Australia, close to the equator).

Pathogenesis

- UV radiation leads to → **formation of pyrimidine dimers in DNA**, which is a type of DNA damage which is responsible for carcinogenicity.
- **DNA damage is repaired by the nucleotide excision repair pathway.**
- With **excessive sun exposure**, the **DNA damage exceeds the capacity of the nucleotide excision repair pathway** and **genomic injury becomes mutagenic and carcinogenic.**
- **Xeroderma pigmentosum**: It is a rare hereditary autosomal recessive disorder characterized by **congenital deficiency of nucleotide excision repair DNA**. These individuals **develop skin cancers** (basal cell carcinoma,

squamous cell carcinoma and melanoma) due to impairment in the excision of UV-damaged DNA.

UV radiation: Induces formation of pyridine dimers in DNA leading to mutations.

Acute leukemia: Most frequent malignant tumor caused by radiation.

Total body radiation: Lymphopenia is the first hematological feature.

Xeroderma pigmentosum is caused due to abnormalities in: Nucleotide excision repair.

Ionizing Radiation

Electromagnetic (X-rays, γ rays) and particulate (α particles, β particles, protons, neutrons) radiations are all carcinogenic.

Ionizing radiation: Damages DNA.

Ionizing radiation: Causes genetic damage by—

1. Chromosomal breakage
2. Translocations
3. Point mutations.

Cancers Produced

- **Medical or occupational exposure**, e.g. leukemia and skin cancers
- **Nuclear plant accidents**: Risk of lung cancers.
- **Atomic bomb explosion**: Survivors atomic bomb explosion (dropped on Hiroshima and Nagasaki) → increased incidence of **leukemias** → mainly acute and chronic myelogenous leukemia after about 7 years. Subsequently, increased mortality due to **solid tumors** (e.g. breast, colon, thyroid and lung).
- **Therapeutic radiation**: (1) **papillary carcinoma of the thyroid** follows irradiation of head and neck and (2) **angiosarcoma of liver** due to radioactive thorium dioxide used to visualize the arterial tree.

Mechanism: Hydroxyl free radical injury to DNA.

Tissues which are relatively resistant to radiation-induced neoplasia: Skin, bone and the gastrointestinal tract.

Neoplasms associated with therapeutic radiation:

1. Papillary carcinoma of thyroid
2. Angiosarcoma of liver.

CLL: Not associated with ionizing radiation.

LABORATORY DIAGNOSIS OF CANCER

Q. Write short note on laboratory diagnosis of cancer.

Confirmation of lesion as neoplastic usually requires cytological and/or histopathological examination of the suspected organ or tissue. Different laboratory methods available for the diagnosis of malignant tumors are:

Morphological Methods

Histopathological specimens: Most commonly used fixative is 10% buffered formaline (formaldehyde).

Histopathological Examination

Histopathological diagnosis is based on the microscopic features of neoplasm and by this method of examination, **accurate diagnosis can be made in majority of cases.**

- **Clinical data**: It should be provided for accurate pathologic diagnosis. Examples:
 - Radiation causes changes in the skin or mucosa mimic changes seen in cancer.
 - Sections taken from the site of a healing fracture can mimic an osteosarcoma.
- **Adequate and representative area of the specimen** should be sent.
- **Proper fixation.**

Diagnosis of neoplasia depends on:

- Clinical investigation
- Imaging
- Laboratory investigations.

Frozen Section

Q. Write short essay/note on frozen section and its uses.

In this method, **tissue is frozen** and **sections are cut by special instrument** called **freezing microtome** or **cryostat**. Its **uses are**:

- **Rapid diagnosis**: Frozen section is used for quick histologic diagnosis (within minutes) and useful for determining the nature of a tumor (benign or malignant) lesion, especially when the **patient is still on the operation table.**
- **Evaluation of the margins of an excised cancer** to know whether excision of the neoplasm is complete.
- **Demonstration of fat** mainly in non-neoplastic lesions.

Various Techniques for Tissue Sampling

- **Needle biopsy:** Using cutting needle, a core of tissue 1–2 mm wide and 2 cm long is obtained. Tissue obtained is small and interpretation may be difficult.
- **Endoscopy biopsy:** It is performed through endoscopy. Usually performed for lesions in gastrointestinal, respiratory, urinary and genital tracts.
- **Incision biopsy:** In this representative tissue sample is obtained by incising the lesions.
- **Excision biopsy:** In this entire abnormal lesion is surgically removed.

Cytological Examination

It is performed on many tissues and usually done for identifying neoplastic cells.

Methods of Obtaining Cells

Q. Write short essay/note on exfoliative cytology.

- Exfoliative cytology:** It is the **study of spontaneously exfoliated (shed) cells** from the lining of an organ into a body cavity.
 - **Sources of exfoliated cells:**
 - **Surface of mucosal or epithelial lining:** Cells may be shed naturally or obtained by artificial exfoliation.
 - ♦ Female genital tract:
 - ◊ Cervix—cells can be obtained by cervical scrape
 - ◊ Vagina
 - ♦ Respiratory tract: Sputum and brush cytology by bronchoscopy
 - ♦ GI tract: Brush cytology by endoscopy
 - ♦ Urinary tract: Voided urine.
 - **Body fluids:** Usually cells are shed naturally into body fluids.
 - ♦ Effusions: Pleural, peritoneal, pericardial
 - ♦ Other fluids: Synovial fluid, CSF and semen.

Principle of exfoliative cytology: Cells normally exfoliate from any surface lining and this exfoliation increases in pathological conditions.

Most common malignant tumor in children: Acute lymphoblastic leukemia.

Most common cause of cancer death in adults: Carcinoma lung.

Q. Write short essay/note on fine-needle aspiration cytology/ FNAC/FNAB (fine needle aspiration biopsy).

- Fine-needle aspiration cytology (FNAC):** It involves aspiration of cells and attendant fluid with a **small-bore needle**. The smears are prepared and stained, followed by microscopic examination of cells. It is **widely used, simple and quick procedure**.
 - **Usual sites:** It is most commonly used for the assessment of **readily palpable superficial lesions** in sites such as the breast (Fig. 7.29), lymph nodes, salivary gland, and thyroid. Presently due to **imaging techniques** this method is also used for lesions in **deep-seated structures** (e.g. pelvic lymph, and lesions in retroperitoneum, liver and pancreas).
 - **Advantages:**
 - ♦ Less invasive and more rapidly performed
 - ♦ Prevents surgery and its associated risks
 - ♦ Extremely reliable and useful.

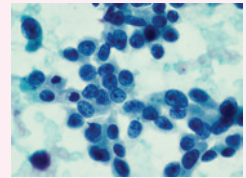


Fig. 7.29: FNAC of infiltrating duct carcinoma of breast

Method of Examination of Cytological Smears

- **Liquid-based cytology (thin prep):** This is a special technique for preparation of samples that provides uniform monolayered dispersion of cells on smears.

Fixatives Used

- For **Pap smears equal parts of ether and 95% ethanol or 95% ethanol alone**
- **Coating fixative** as aerosol sprays or with dropper to the surface of a freshly prepared smears

Pap smears are fixed immediately in fixative when smear is still wet and dry smears are fixed after the smear is air dried.

Staining of Smears

Cytological smears can be stained by:

- Papanicolaou stain is used for wet smears.
- Hematoxylin and eosin (H&E) stain
- Romanowsky stain: It includes- May-Grunwald-Giemsa (MGG) stain, Leishman stain and Wright's stain.

Cytological Characteristics of Cancer Cells

Cancer cells have decreased cohesiveness and show cellular features of **anaplasia**. Cytologically, differentiation can be made between normal, dysplastic, carcinoma in situ and malignant cells.

Disadvantages of Cytological Examination

- **Diagnosis is based on the features of individual cells** or a clump of cells, without the supporting evidence of loss of orientation.
- The **invasion** which is diagnostic of malignant tumor under histology **cannot be assessed** by cytology.

Histochemistry and Cytochemistry

These are stains, which identify the chemical nature of cell contents or their products. H&E staining cannot demonstrate certain specific substances/constituents of cells. This requires some special stains. Common histochemical and cytochemical stains useful in diagnosis of tumors are listed in Table 7.14.

Immunohistochemistry

Q. Write short note on immunohistochemistry and its role in the diagnosis of tumors.

It is an immunological method of identifying the antigenic component in the cell or one of its components by using specific antibodies. It is widely used in the diagnosis or management of malignant neoplasms.

Uses of Immunohistochemistry

Uses of immunohistochemistry:

1. Categorization of undifferentiated cancers
 2. Determine the origin of poorly differentiated metastatic tumor
 3. Select the mode of treatment and to know the prognosis
- **To categorize undifferentiated cancers:** Many malignant tumors of diverse origin resemble each other and are difficult to distinguish on routine hematoxylin and eosin (H&E) sections.

TABLE 7.14: Common histochemical and cytochemical stains useful in diagnosis of tumors

Chemical substance	Name of the stain
Basement membrane/ collagen	Periodic Acid Schiff (PAS)
	Reticulin
	Masson trichrome
	Van Gieson
Glycogen	PAS with diastase
Mucin	Combined Alcian blue-PAS
	Mucicarmine
Cross-striation	Phosphotungstic acid hematoxylin (PTAH)
Enzymes	Myeloperoxidase
	Acid phosphatase
	Alkaline phosphatase

- Example: Few anaplastic carcinomas, lymphomas, melanomas and sarcomas may look almost similar. They should be accurately diagnosed because of their different modes of treatment and prognosis.

- ♦ **In poorly differentiated carcinoma intermediate filaments** (e.g. cytokeratins) shows positivity (Table 7.15).
- ♦ **Malignant melanomas when unpigmented** (amelanotic melanoma) appear similar to other poorly differentiated carcinomas. They express **HMB-45** and **S-100 protein**, but negative for cytokeratins.
- ♦ **Desmin** is found in neoplasms of **muscle cell origin**.
- **To determine the origin of poorly differentiated metastatic tumors:** It may be determined by using tissue-specific or organ-specific antigens (Table 7.16).
- **For prognosis or to select the mode of treatment:**
 - Identification of **hormone** (estrogen/progesterone) **receptors in breast cancer** cells is of **prognostic and therapeutic value**. These cancers respond well to antiestrogen therapy and have a better prognosis.
 - Breast cancers with **ERBB2 protein (HER2/NEU)** **positivity have a poor prognosis**.

Carcinoma: Cytokeratin +ve.

Malignant melanoma: HMB-45 +ve (more specific) and S100 +ve (more sensitive) after <S100+ve>.

Immunohistochemical Markers

Apart from the various immunochemical markers mentioned above, other markers useful are as follows:

- **Neuroendocrine tumors** show positivity for cytokeratins like carcinomas, but they can be identified by their contents, namely:
 - Chromogranins (proteins found in neurosecretory granules)
 - Neuron-specific enolase (NSE)
 - Synaptophysin.
- **Soft tissue sarcomas:** They show intermediate filament positivity
 - **Vimentin**
 - **Desmin** positive in smooth or striated muscle fibers
 - **Muscle-specific actin** marker for muscle tissue.
- **Neurofilament proteins: Marker for tumors of neurons, neuroblastomas and ganglioneuroma.**
- **Neuron-specific enolase (NSE)** in neuroblastomas.
- **Glial fibrillary acidic protein (GFAP)**, also intermediate filament expressed in **glial cell neoplasms**.
- **Malignant lymphomas:** Generally positive for **leukocyte common antigen (LCA, CD45)**. Markers for lymphomas

TABLE 7.15: Intermediate filaments and their use in diagnosis of neoplasms

Type of intermediate filament	Normal tissue expression	Diagnostic usefulness
1. Cytokeratin	All epithelial cells	Carcinoma
2. Vimentin	Mesenchymal cells	Sarcoma
3. Desmin	Muscle cells	Tumors of muscle (e.g. rhabdomyosarcoma)
4. Glial fibrillary acid protein (GFAP)	Glial cells	Glial tumors (e.g. astrocytoma)
5. Neurofilament (NF)	Neurons and neural crest derivatives	Neural tumors (e.g. neuroblastoma)

TABLE 7.16: Lineage-associated immunohistochemical markers useful in establishing the origin of a poorly carcinoma

Lineage-associated markers	Associated cancer
Prostate-specific antigen (PSA) and prostate-specific acid phosphatase (PSAP)	Prostatic carcinoma
Carcinoembryonic antigen (CEA)	Colonic carcinoma
Thyroglobulin	Thyroid carcinoma
CA 125	Ovarian cancers
Nuclear receptors for estrogen and progesterone	Carcinoma of breast

and leukemias are called cluster designations (CDs) and useful to differentiate T and B lymphocytes, monocytes, and granulocytes and the mature and immature variants of these cells.

- **Vascular tumors** derived from endothelial cells, include benign hemangiomas, and malignant hemangiosarcomas and are positive for factor **VIII-related antigen** or certain lectins.
- **Proliferating cells:** Cells in cell cycle show positivity for **Ki-67** and proliferating cell nuclear antigen (**PCNA**).

BCL2 is a marker for: Follicular lymphoma.

Neuroendocrine tumors:

- Chromogranin +ve
- NSE +ve
- Synaptophysin +ve.

Malignant lymphoma: Leukocyte common antigen (LCA, CD45) +ve.

Soft tissue sarcoma:

- Vimentin +ve
- Desmin +ve in tumors of smooth or striated muscle.

Electron Microscopy

Electron microscopy: Fixative used is glutaraldehyde

It helps in the **diagnosis of poorly differentiated/undifferentiated cancers**, which cannot identify the origin by light microscopy. Example: Carcinomas show desmosomes and specialized junctional complexes, structures which are not seen in sarcomas or lymphomas.

Flow Cytometry

Immunohistochemistry and flow cytometry: Help in the diagnosis and classification of neoplasms.

Q. Write short note on modern techniques in tumor diagnosis.

It quantitatively measures various individual cell characteristics, such as membrane antigens and the DNA content of tumor cells. Flow cytometry is useful for identification and **classification of tumors of T and B lymphocytes** and mononuclear-phagocytic cells.

Circulating Tumor Cells

Detection, quantification, and characterization of rare solid tumors cells (e.g. carcinoma, melanoma) circulating in the blood is emerging as a diagnostic modality though presently in research stage. Few latest devices detect three-dimensional flow cells coated with antibodies specific for tumor cells of interest (e.g. carcinoma cells) in the blood. It will be useful for early diagnosis, to assess the risk of metastasis and assess the response of tumor cells to therapy.

Tumor Markers

Q. Write short note on tumor markers.

Q. List tumor markers giving one example for each.

Tumor markers are **products of malignant tumors** that can be detected in the cells themselves or in blood and body fluids.

Tumor markers: Products of malignant tumors.

Usefulness

- **Detection of cancer**, e.g. **PSA** is the most common and useful tumor markers used to screen **prostatic adenocarcinoma**. High levels of PSA are found in the blood of prostatic carcinoma patients but it also may be elevated in benign prostatic hyperplasia.
- **Determine the effectiveness of therapy.**
- **Detection of recurrence.**

Types of markers (Table 7.17): These may be tumor-associated hormones, oncofetal antigens, specific proteins, mucin and glycoproteins, enzymes and molecular markers.

PSA is specific for prostatic diseases but not specific for prostatic cancer. This has both low sensitivity and low specificity.

TABLE 7.17: Common tumor markers

Q. Write short note on tumor markers for choriocarcinoma

Q. Write short note on carcinoembryonic antigen.

Q. Write short note on alpha fetoprotein.

Tumor marker	Associated tumors
1. Hormones	
– Human chorionic gonadotropin (hCG)	Trophoblastic tumors, nonseminomatous tumors of testis
– Calcitonin	Medullary carcinoma of thyroid
– Catecholamine	Pheochromocytoma
– Ectopic hormones	Paraneoplastic syndromes (Table 7.18)
2. Oncofetal Antigens	
– α -Fetoprotein (AFP)	Cancer of liver, nonseminomatous germ cell tumors of testis
– Carcinoembryonic antigen (CEA)	Carcinomas of the colon, pancreas, lung and stomach
3. Mucins and Other Glycoproteins	
– CA-125	Ovarian cancer
– CA-19-9	Colon cancer, pancreatic cancer
– CA-15-3	Breast cancer
4. Isoenzymes	
– Prostatic acid phosphatase (PAP)	Prostate carcinoma
– Neuron-specific enolase (NSE)	Small-cell carcinoma of lung, neuroblastoma
5. Specific Proteins	
– Immunoglobulins	Multiple myeloma and other gammopathies
– Prostate-specific antigen (PSA)	Prostate carcinoma
6. New Molecular Markers	
– p53, APC, RAS mutants in stool and serum	Carcinoma colon
– p53 and RAS mutants in sputum and serum	Lung cancer

Elevated AFP:

- Hepatocellular carcinoma
- Germ cell tumor
- Cirrhosis

CA.125 is associated with: Ovarian cancer

Molecular Diagnosis

Molecular diagnosis can be done by different techniques such as FISH technique and PCR (polymerase chain reaction) analysis.

a. Diagnosis of cancer:

- **Monoclonal (malignant) vs polyclonal (benign):** To differentiate benign (polyclonal) proliferations of T- or B-cells from malignant (monoclonal) proliferations.
- **Chromosomal alterations:** Many **hematopoietic neoplasms** (leukemias and lymphomas) and few solid tumors (e.g. Ewing sarcoma) are characterized by particular translocations that can be detected by FISH technique or by PCR analysis.

- b. **Prognosis of cancer:** Certain genetic alterations are of prognostic value. They can be detected by routine cytogenetics and also by FISH or PCR assays. Example of poor prognostic feature is amplification of the *N-MYC* gene and deletions of 1p in neuroblastoma and amplification of *HER-2/Neu* in breast cancer.
- c. **Detection of minimal residual disease:** PCR can detect minimal residual disease or the onset of relapse in patients who are treated for leukemia or lymphoma. For example, detection of *BCR-ABL* transcripts in treated patients with CML.
- d. **Detection of hereditary predisposition to cancer:** Germ-line mutations in many tumor suppressor genes are associated with increased risk for specific cancers. This will help in prophylactic surgery, and counseling of relatives at risk. For example, *BRCA1*, *BRCA2* and the *RET* proto-oncogene.
- e. **For therapeutic decision:** It is useful in target therapy. **Molecular profiles of tumors:** Present methods like DNA microarray technology can measure the expression **single gene to all genes** in the genome instead of only one gene at a time.

CLINICAL ASPECTS OF NEOPLASIA

Q. Clinical features of malignant tumors.

Both benign and malignant tumors may produce clinical features by its various effects on host.

Local Effects

These are due to encroachment on adjacent structures.

- **Compression:** For example, adenoma in the ampulla of Vater causing obstruction of biliary tract.
- **Mechanical obstruction:** It may be caused by both benign and malignant tumors. Example: Tumors may cause obstruction or intussusception in the GI tract.
- **Endocrine insufficiency:** It is caused due to destruction of an endocrine gland either due to primary or metastatic cancer.
- **Ulceration, bleeding and secondary infections:** It may develop in benign or malignant tumors in the skin or mucosa of the GI tract. Example:
 - Melena (blood in the stool) in neoplasms of the gut
 - Hematuria in neoplasms of the urinary tract.
- **Rupture or infarction of tumor.**

Functional Effects

Functional effects of tumor:

- Production of hormones
- Paraneoplastic syndrome
- Fever

These include:

- **Hormonal effects:** It may be observed both in benign and malignant tumors of endocrine glands. Example: β -cell adenoma of the pancreas may produce insulin \rightarrow to cause fatal hypoglycemia.
- **Paraneoplastic syndromes:** Nonendocrine tumors may secrete hormones or hormone-like substances and produce paraneoplastic syndromes (explained below).
- **Fever:** It is most commonly associated with Hodgkin disease, renal cell carcinoma and osteogenic sarcoma. Fever may be due to release of pyrogens by tumor cells or IL-1 produced by inflammatory cells in the stroma of the tumor.

Tumor Lysis Syndrome

- It is a group of metabolic complications that can occur after treatment for leukemias such as acute **lymphoblastic leukemia (ALL)**, **chronic lymphocytic leukemia (CLL)**; lymphomas such as Burkitt lymphoma, and uncommonly solid tumors.
- It is caused by **breakdown products of tumor cells following chemotherapy or glucocorticoids or hormonal agent (tamoxifen)**.
- The killed tumor cells **release intracellular ions and large amounts of metabolic byproducts into systemic circulation**.
- **Metabolic abnormalities** include:
 - **Hyperuricemia:** Due to increased turnover of nucleic acids.
 - **Hyperkalemia:** Due to release of the most abundant intracellular cation potassium.
 - **Hyperphosphatemia:** Due to release of intracellular phosphate.
 - **Hypocalcemia:** Due to complexing of calcium with elevated phosphate.
 - **Lactic acidosis.**
 - **Hyperuricemia:** It can cause uric acid precipitation in the kidney resulting in **renal failure**.

Tumor lysis syndrome: Associated with hypocalcemia and NOT hypercalcemia.

Cancer Cachexia (Wasting)

Q. Write short note on cachexia

It is defined as **progressive weight loss accompanied by severe weakness, anorexia and anemia developing in patients with cancer**.

- **Mechanism:** It is poorly understood and may be due to TNF and other cytokines, like IL-1, interferon- γ ,

and leukemia inhibitory factor. They may be produced by macrophages in the tumor or by the tumor cells themselves.

Cachexia: TNF- α plays an important role.

Cancer cachexia: Progressive weight loss accompanied by severe weakness, anorexia and anemia.

PARANEOPLASTIC SYNDROMES

Q. Write short note on paraneoplastic syndrome.

Malignant tumors invade local tissue, produce metastasis and can produce a variety of products that can stimulate hormonal, hematologic, dermatologic and neurologic responses.

Definition: Paraneoplastic syndromes are **symptom complexes in cancer patients** which are **not directly related to mass effects or invasion or metastasis or by the secretion of hormones indigenous to the tissue of origin.**

Frequency: Though they occur in 10–15% of patients, it is important because:

1. **May be the first manifestation** of an occult neoplasm.
2. **May be mistaken for metastatic disease** leading to inappropriate treatment.
3. May present clinical problems which **may be fatal.**
4. Certain tumor products causing paraneoplastic syndromes may be **useful in monitoring recurrence** in patients who had surgical resections or are undergoing chemotherapy or radiation therapy.

Some paraneoplastic syndromes, their mechanism and common cancer causing them are listed in Table 7.18.

PROGNOSIS

Q. Write short note on prognostic factors of malignant tumors.

The prognosis of malignant tumors vary and is determined partly by the characteristics of the tumor cells (e.g. growth rate, invasiveness) and partly by the effectiveness of therapy.

Prognostic Indices

Prognosis of tumor depends on:

1. Histological type
2. Grade
3. Stage.

Prognosis and the treatment of a malignant tumor depend on:

1. **Tumor type:** It is usually identified from the growth pattern of the tumor and its origin by only histopathological examination.
 - **Prognosis depends on the histological type** (e.g. squamous cell carcinoma, melanoma, adenocarcinoma, leiomyosarcoma).
 - Some tumors like lymphomas require further subclassification into Hodgkin and non-Hodgkin's lymphoma, each of which is then further subclassified by the cell type.
2. **Grading of malignant tumors:** It is done by histological examination and is mainly based on the **degree of differentiation** of the tumor cells.
 - In general, there is a **correlation between histologic grade and biologic behavior.**
 - Most grading systems classify tumors into three or four grades of increasing malignancy. Low-grade tumors are well-differentiated; high-grade ones tend to be anaplastic.
 - **Shortcomings:** (1) **Less correlation with behavior:** In general, in soft-tissue sarcomas, grading is of less clinical value than staging; (2) **subjective:** Grading is subjective and the degree of differentiation can vary in different areas of the same tumor.

Grading of tumor depends on the degree of differentiation.

3. **Staging of tumors:** It refers to the extent of spread of a malignant tumor and is independent of grading. The **mode of treatment is determined by the stage of a cancer** than by its grade.
 - **Criteria:** Staging requires **both histopathological examination** of the resected tumor and **clinical assessment** of the patient [including additional non-invasive techniques like computed tomography (CT), magnetic resonance imaging (MRI) and positron emission tomography (PET)].
 - The **criteria used** for staging vary with different **organs.** Commonly the staging of cancers is based on:
 - **Size and extent of local growth of the primary tumor:** For example, in colorectal cancer, the tumor which has penetrated into the muscularis and serosa of the bowel is associated with a poorer prognosis than with a tumor restricted to superficial mucosa/submucosa.
 - **Extent of spread to regional lymph nodes:** Presence of lymph node metastases indicate poor prognosis than without lymph node involvement.
 - **Presence of or absence of blood-borne (distant) metastases:** The presence of blood-borne distant metastases is bad prognostic sign and is a contraindication to surgical intervention other than for palliative measures.

TABLE 7.18: Paraneoplastic syndromes

Q. Write short note on tumors which produce paraneoplastic syndromes.

Q. Write short note on paraneoplastic syndromes produced by oat cell carcinoma of lung.

Q. Write short note on paraneoplastic syndromes produced by renal cell carcinoma.

<i>Clinical syndromes</i>	<i>Cause/mechanism</i>	<i>Example of associated cancer</i>
1. Endocrinopathies		
Cushing syndrome	ACTH or ACTH-like substance	Small-cell carcinoma of lung
Syndrome due to inappropriate antidiuretic hormone secretion (SIADH)	Antidiuretic hormone or atrial natriuretic hormones	Small-cell carcinoma of lung
Hypercalcemia	Parathyroid hormone-related protein (PTHrP), TGF- α , TNF, IL-1	Squamous cell carcinoma of lung Renal carcinoma
Carcinoid syndrome	Serotonin, bradykinin	Bronchial carcinoid
Hypoglycemia	Insulin or insulin-like substance	Fibrosarcoma
Polycythemia	Erythropoietin	Renal carcinoma, hepatocellular carcinoma
2. Neurologic (neuromyopathic) syndromes		
Myasthenia	Immunological	Bronchogenic carcinoma
3. Cutaneous syndromes		
Acanthosis nigricans	Immunological; secretion of epidermal growth factor	Carcinoma of stomach, lung and uterus
Dermatomyositis	Immunological	Bronchogenic, breast carcinoma
Exfoliative dermatitis	Immunological	Lymphoma
4. Changes in osseous, articular and soft-tissue		
Hypertrophic osteoarthropathy and clubbing of the fingers	Not known	Bronchogenic carcinoma
5. Vascular and hematologic syndromes		
Venous thrombosis (Trousseau syndrome)	Tumor products like mucins which activate clotting	Pancreatic carcinoma Bronchogenic carcinoma
Disseminated intravascular coagulation	Procoagulant substance: Cytoplasmic granules (e.g. acute promyelocytic leukemia cells) or mucus (adenocarcinomas)	Acute promyelocytic leukemia, prostatic adenocarcinomas
Nonbacterial thrombotic endocarditis	Hypercoagulability	Advanced mucus secreting adenocarcinomas
6. Renal syndromes		
Nephrotic syndrome	Tumor antigens, immune complexes	Various cancers
7. Amyloidosis		
Primary amyloidosis	Immunological (AL protein)	Multiple myeloma
Secondary amyloidosis	AA protein	Renal cell carcinoma and other solid tumors

Abbreviations: ACTH, adrenocorticotrophic hormone; IL, interleukin; TGF, transforming growth factor; TNF, tumor necrosis factor.

Syndrome of inappropriate ADH (SIADH): Most common cause is oat cell carcinoma of lung.

TNM Staging Systems

Q. Grading and staging of cancer.

It is the cancer staging system widely used and it varies for each specific form of cancer. Its general principles are:

TNM staging system widely used.

- **T** refers to the **size of the primary tumor**.
 - It is suffixed by a number which indicates the size of the tumor or local anatomical extent. The number varies according to the organ involved by the tumor. With increasing size, the primary lesion is characterized as T1 to T4. T0 is used to denote an in situ lesion.

- **N** refers to **lymph node status**.
 - It is suffixed by a number to indicate the number of lymph regional nodes or groups of lymph nodes showing metastases.
 - N0 would mean no nodal involvement, whereas N1 to N3 would denote involvement of an increasing number and range of nodes.
- **M** refers to the presence and anatomical extent of **distant metastases**.
 - M0 signifies no distant metastases, whereas M1 indicates the presence of metastases.

TNM staging

- T=size of primary tumor
- N=lymph node status
- M=Metastatic status.

Genetic Disorders

Genetics is the study, which deals with the science of genes, heredity and its variation in living organisms.

GENES

Definition: Gene is defined as a segment of deoxyribonucleic acid (DNA) which carries the genetic information. Gene is the basic physical and functional unit of heredity. DNA has also segments which do not contain genes.

The human genome contains about **19,000 genes** and each gene varies in size.

Structure of Gene (Fig. 8.1)

Each gene consists of a specific sequence of nucleotides. **Genes may be silent or active.** When active, the **genes direct the process of protein synthesis.** Genes do not code for proteins directly but **by means of a genetic code.** The **genetic code** consists of a sequence codeword called **codons**. A codon for an amino acid consists of a sequence of three nucleotide base pairs called a **triplet codon**.

Regions of Gene

- **Initiator and stop codons:** The boundaries of a gene are known as **start and stop codons**. The start codon tells

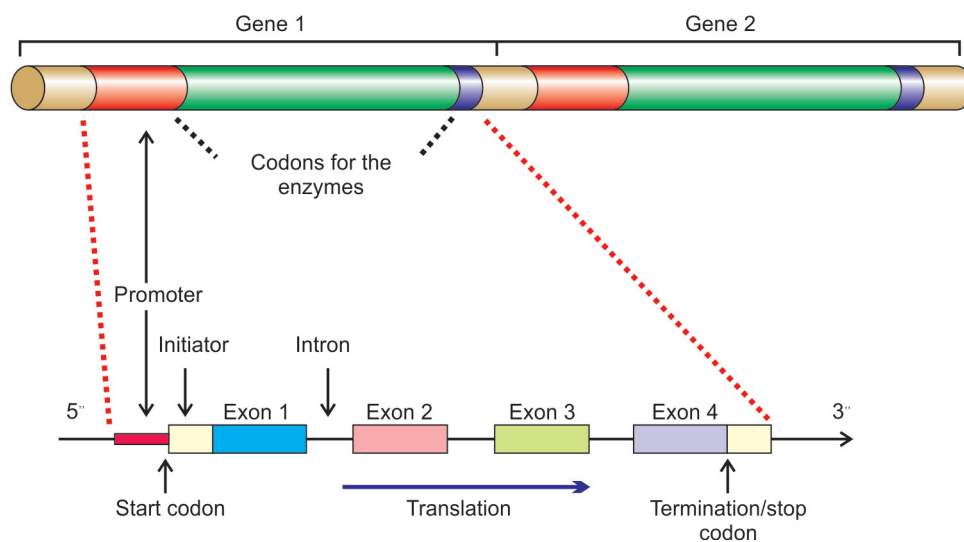


Fig. 8.1: Diagrammatic structure of gene. Start and termination codons mark the limits of the gene. The coding portion of the gene is exons (four in this example), and interspersed with introns

when to begin protein production and stop (termination) codons tells when to end the protein production.

- **Coding region:** The **nucleotide sequence between the start and stop codons** is the core region known as **coding region**. This region is divided into two main segments namely, exons and introns. Most of the genes contain both exons and introns, the number of which varies with different genes.
 - **Exons:** This region codes for producing a protein.
 - **Introns:** These are the regions between exons and do not code for a protein (**noncoding region**).
- **Regulatory regions:** These are also noncoding regions which control gene expression.
 - **Promoters** are regions which bind to transcription factors, either strongly or weakly.
 - **Enhancers** are regions which can enhance the effect of a weak promoter.
 - **Silencers** are regulatory regions that can inhibit transcription.

Genotype: Genetic makeup of an individual.

Phenotype: Manifested physical feature.

Allele: Alternate form of gene coding for different forms of character.

Normal gene has 2 alleles.

Homozygous: Two alleles code for same trait.

Heterozygous: Two alleles code for different traits.

CLASSIFICATION OF GENETIC DISORDERS

Genetic disorders are classified into three major categories (Box 8.1).

MUTATIONS

Single gene disorders result from mutations in single gene.

Definition: A mutation is defined as a **permanent change in the genetic material (DNA) which results in a disease**. The term mutation was coined by Muller in 1927.

Causes

- **Spontaneous mutation:** Majority of mutations occur spontaneously due to errors in DNA replication and repair.
- **Induced mutation:** Mutations can be caused due to exposure to mutagenic agents like chemicals, viruses, and ultraviolet or ionizing radiation.

Polymorphism: If the **genetic material change/variant does not cause obvious effect upon phenotype, it is termed**

BOX 8.1: Classification of genetic disorders

- A. Single-gene or monogenic disorders/Mendelian disorders
 - Autosomal dominant
 - Autosomal recessive
 - X-linked dominant
 - X-linked recessive.
- B. Cytogenetic disorders-chromosomal disorders (aberrations/abnormalities)
 - Numerical aberrations aneuploidy (trisomy, monosomy), polyploidy, and mosaicism
 - Structural aberrations
 - Translocations
 - ♦ Balanced reciprocal translocations
 - ♦ Robertsonian translocations
 - Inversion
 - Isochromosome
 - Ring chromosome
 - Deletions
 - Insertions.
- C. Complex/multifactorial multigenic/polygenic disorders
 - Diabetes mellitus
 - Hypertension.

as polymorphism. A polymorphism is defined as genetic variation that exists in population with a frequency of > 1%.

Classification of Mutations

Depending on the cell affected: Mutations can affect either somatic cells or germ cells.

- **Germ cell mutations:** Mutations that affect the germ cells are transmitted to the progeny/ descendants and can produce inherited/hereditary diseases.
- **Somatic cell mutations:** Mutations involving the somatic cells can produce cancers and some congenital malformations. These mutations are not inherited and do not cause hereditary diseases are known as de novo mutations.

Structural Chromosomal Mutations

The rearrangement of genetic material causes structural change. Structural mutations may be (1) visible during karyotyping (refer page 224–225) or (2) submicroscopic (minute/subtle changes).

Minute/Subtle Changes

The **submicroscopic gene mutations** can result in partial or complete deletion of a gene or more often, a single nucleotide base.

A. Point Mutation

Q. Write short note on point mutation.

It is characterized by replacement of one **nucleotide base** by a **different nucleotide base within a gene**.

1. **Within coding sequences:** Majority of point mutation occurs in the coding region of a gene.
 - **Missense mutations:** If point mutations change the genetic code, it may code for a different amino acid and protein.
 - **Conservative missense mutation:** In this type, the substituted amino acid produces **only little change in the function of the protein**.
 - **Nonconservative missense mutation:** In this type, normal amino acid is replaced by very different amino acid and result in **change in function of protein**. Example, sickle cell anemia in which mutation affect the β -globin chain of hemoglobin (Fig. 8.2). In this, the nucleotide triplet CTC, which encodes glutamic acid, is changed to CAC, which encodes valine. This single amino acid substitution changes the properties of hemoglobin, giving rise to sickle cell anemia.
 - **Nonsense mutation (stop codon):** In this type, point mutation changes an amino acid codon to a **premature**

termination codon. Example, in β -globin chain, a point mutation affecting the codon for glutamine (CAG) creates a stop codon (UAG) if U replaces C (Fig. 8.3). This change leads to premature termination of β -globin gene translation \rightarrow deficiency of β -globin chains \rightarrow no synthesis of hemoglobin A. It produces a severe form of anemia called β^0 -thalassemia.

Point mutation: Mutation involving a change in a single nucleotide base within a gene.

Point mutations:

- Silent mutations: Altered DNA codes same amino acid
- Missense mutations: Altered DNA codes different amino acid
- Nonsense mutation: Altered DNA codes for stop codon and causes premature termination of protein synthesis.

Missense mutations: Sickle cell anemia/trait.

Nonsense mutation with stop codon: β -thalassemia major.

B. Mutations within Noncoding Sequences

Mutations may also involve these noncoding regions of gene. Point mutations or deletions involving these regulatory regions may lead to either marked reduction in or total lack of transcription. Example, certain hereditary anemias.

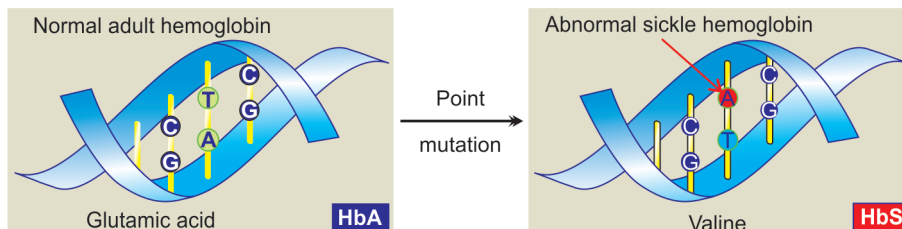


Fig. 8.2: Nonconservative missense point mutation in sickle cell anemia. When adenine replaces thymidine, the amino acid valine replaces glutamic acid in the sixth position of β -globin chain of hemoglobin and give rise to abnormal sickle hemoglobin.

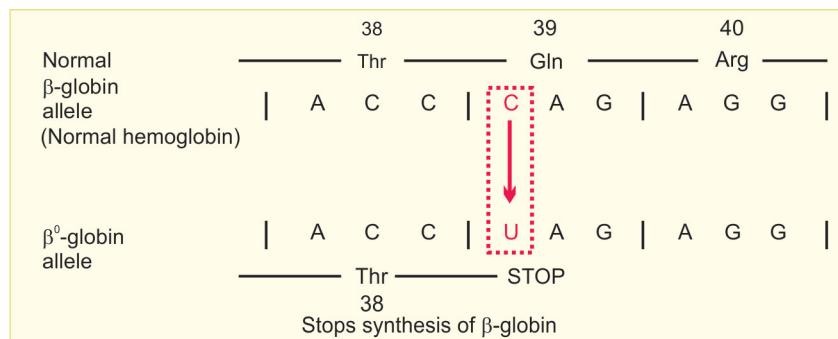


Fig. 8.3: Nonsense point mutation leading to premature chain termination. A point mutation (C replaced by U) in codon 39 changes glutamine (Gln) codon to a stop codon. This stops the synthesis of protein at amino acid 38

C. Frame Shift Mutation

This may occur due to **insertion or deletion of one or more nucleotides** in the coding regions. If the number of nucleotide bases inserted or deleted is not a multiple of 3, the code will be changed (Fig. 8.4A). This leads to alterations in the reading frame of the DNA strand; hence they are known as frameshift mutation. If the number of base pairs involved is three or a multiple of three, frameshift does not occur. This may synthesize an abnormal protein lacking or gaining one or more amino acids. When deletions involve a large segment of DNA, the coding region of a gene may be entirely removed (Fig. 8.4B).

D. Trinucleotide Repeat Mutation

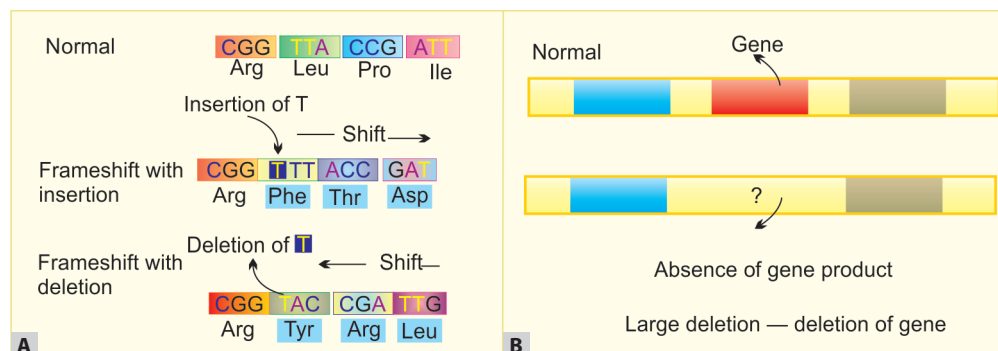
The DNA contains several repeat sequences of three nucleotides (**trinucleotide**). When they are repeated directly adjacent to each other (one right after the other), they are known as **tandem repeats** (Fig. 8.5). When the repetitive trinucleotide sequences reach above a particular threshold,

they can expand (amplify) or contract. The amplification is more common. These trinucleotide-repeat mutation are dynamic (i.e. the degree of amplification increases during gametogenesis).

Functional Effect

Mutations in DNA can lead to either change in the amino acid sequence of a specific protein or may interfere with its synthesis. The consequences vary from those without any functional effect to those which have serious effects.

- **Loss-of-function (LOF) mutations:** These mutations cause the reduction or loss of normal function of a protein. It is usually due to deletion of the whole gene but may also occur with a nonsense or frameshift mutation.
- **Gain-of-function mutations:** These are usually due to missense mutations. In gain-of-function mutation, the protein function is altered in a manner that results in a change in the original function of the gene.
- **Lethal mutations:** These lead to death of the fetus.



Figs 8.4A and B: Frameshift mutations. (A) Insertion or deletion of nucleotide bases if not a multiple of 3, the code will be changed; (B) Deletions involving a large segment of DNA in the coding region may entirely remove the gene

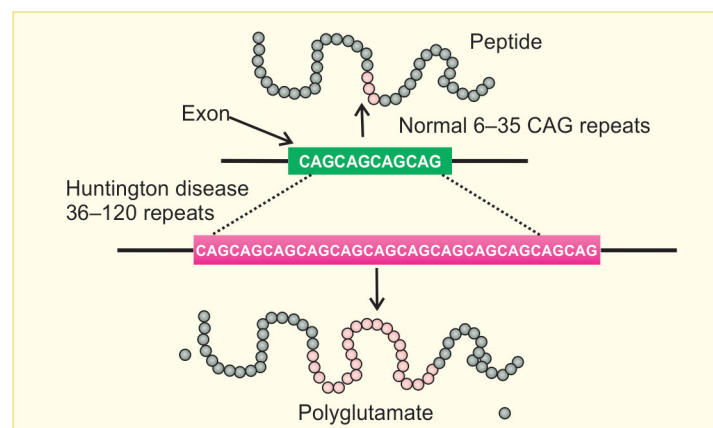


Fig. 8.5: Trinucleotide repeat disorder, e.g. Huntington disease. It results from expansion of a CAG triplet repeat from a normal number of 6 to 35 repeats to greater than 36 repeats. This results in expansion of a polyglutamine sequence in the corresponding protein

MENDELIAN DISORDERS/SINGLE-GENE OR MONOGENIC DISORDERS

Mendelian disorders:

1. Genetic disorders due to mutations in single gene.
2. Defective gene may be in the autosome or sex chromosome.

These genetic disorders result from mutations in single gene.

General Features

- **Location of defective gene:** It is on autosomes (autosomal inheritance) or the sex chromosomes (sex-linked inheritance).
- **Dominant versus recessive gene:** Genes are inherited in pairs—one gene from each parent. However, the inheritance may not be equal, and **one gene may overpower the other** in their coded characteristic. The **gene that overshadows the other is called the dominant gene; the overshadowed gene is the recessive one.**
- **Homozygote versus heterozygote:** In some autosomal mutations, the disease is partially expressed in the heterozygote and fully expressed in the homozygote, e.g. sickle cell anemia.
- **Codominant inheritance:** Sometimes both of the alleles of a gene pair contribute to the expression of phenotype. It is called codominance, e.g. blood group antigen.

Inheritance pattern of ABO blood group system: Codominant.

Patterns of inheritance for Mendelian disorders:

1. Autosomal dominant
2. Autosomal recessive
3. X-linked dominant
4. X-linked recessive.

Autosomal Dominant Pattern of Inheritance

General Features

- **Location of mutant gene:** It is on **autosomes**.
- **Required number of defective genes:** Only **one copy**.
- **Sex affected:** Both **males and females** are **equally** affected.
- **Pattern of inheritance:**
 - **Every affected individual has** at least one **affected parent**.
 - Normal members of a family do not transmit the disorder to their children.

- **Risks of transmission to children (offspring):** Affected males and females have an equal risk of passing on the disorder to children.

• Additional properties

- **Penetrance:** It is the percentage of individuals (with mutation) having clinical symptoms.
 - ♦ With **complete penetrance**, **all individuals show clinical symptoms**
 - ♦ With **reduced penetrance**, **only some individuals show disease**
 - ♦ In **nonpenetrance**, individuals may **not show any symptoms**.
- **Variable expressivity** (qualitatively or quantitatively) of disorder is the term used for variable expression among individuals (even within the same family).
- **Delayed onset:** Symptoms and signs may be delayed and may not appear until adulthood. Example, Huntington's disease.

Penetrance: Percentage of individuals (with mutation) having clinical symptoms.

Autosomal dominant: With reduced penetrance only some individuals show disease.

Autosomal dominant:

- Expression in heterozygous state
- Males and females equally affected
- Both sexes can transmit the disorder.

Table 8.1 shows common autosomal dominant disorders.

TABLE 8.1: Example of autosomal dominant disorders

Q. Name autosomal dominant disorders.

System affected	Examples
Nervous system	<ul style="list-style-type: none"> • Huntington's disease • Neurofibromatosis • Tuberous sclerosis
Musculoskeletal system	<ul style="list-style-type: none"> • Marfan syndrome • Osteogenesis imperfecta • Achondroplasia
Hematopoietic system	<ul style="list-style-type: none"> • Hereditary spherocytosis • von Willebrand disease
Renal system	<ul style="list-style-type: none"> • Polycystic kidney disease
Gastrointestinal system	<ul style="list-style-type: none"> • Familial polyposis coli
Metabolic disorders	<ul style="list-style-type: none"> • Familial hypercholesterolemia

Autosomal Recessive Pattern of Inheritance

Most common type of Mendelian disorder is autosomal recessive type.

Autosomal recessive disorders constitute the **largest group of Mendelian disorders**.

General Features

Autosomal recessive:

- Disease develops when both copies of gene are mutated
- Males and females equally affected.
- **Location of mutant gene:** It is on **autosome**.
- **Required number of defective gene:** **Symptoms of the disease appear only when** an individual has **two copies** (both alleles at a given gene locus) of the mutant gene. **When an individual has one mutated gene and one normal gene**, this heterozygous state is called as a **carrier**.
- **Pattern of inheritance:** For a child to be at risk, **both parents must be having at least one copy of the mutant gene**. For example, all inborn errors of metabolism.
- **Sex affected:** **Females and males are equally affected**.
- **Consanguineous marriage:** It is a common predisposing factor.
- **Risks of transmission:** Siblings have one in four chance of having the trait (i.e. the recurrence risk is 25% for each birth).
- **Expression of disease:** It is more uniform than in autosomal dominant disorders.
- **Penetrance:** **Complete penetrance** is common.
- **Onset:** It frequently manifest early in life.

Autosomal recessive disorders: Include almost all inborn errors of metabolism.

Autosomal recessive disorder: Both parents must have a mutant gene.

Carrier: Heterozygous state in which an individual has one mutated gene and one normal gene.

Examples of autosomal recessive disorders are shown in Table 8.2.

X-linked Pattern of Inheritance

Male to male transmission is not seen in X-linked dominant disease.

Almost all sex-linked Mendelian disorders are X-linked. Males with mutations involving the Y-linked genes are

TABLE 8.2: Examples of autosomal recessive disorders

Q. Name autosomal recessive disorders.

System affected	Examples
Inborn errors of metabolism	<ul style="list-style-type: none"> • Phenylketonuria • Galactosemia • Cystic fibrosis • Homocystinuria • Hemochromatosis • Lysosomal storage diseases (page 229) • Glycogen storage diseases • Wilson disease • α1-Antitrypsin deficiency
Hematopoietic system	<ul style="list-style-type: none"> • Sickle cell anemia • Thalassemias
Skeletal system	<ul style="list-style-type: none"> • Alkaptonuria
Nervous system	<ul style="list-style-type: none"> • Friedreich ataxia
Endocrine system	<ul style="list-style-type: none"> • Congenital adrenal hyperplasia

usually infertile, and hence there is no Y-linked inheritance.

Expression of an X-linked disorder is different in males and females.

- **Females:** The **clinical expression** of the X-linked disease is **variable**, depending on whether it is dominant or recessive. **Females are rarely affected by X-linked recessive diseases**; however they are affected by X-linked dominant disease.
- **Males:** Mutation affecting X chromosome is **fully expressed** even with one copy, regardless of whether the disorder is dominant or recessive.

X-linked Recessive Traits

X-linked recessive inheritance: Asymptomatic female carrier transmits mutant gene to 50% of male children.

General Features

- **Location of mutant gene:** It is on the **X chromosome** and there is **no male-to-male transmission**.
- **Required number of defective gene:** **One copy** for the manifestation of disease **in males**, but **two copies** are needed **in females**.
- **Sex affected:** Males are more frequently affected than females; daughters of affected male are all asymptomatic carriers. Affected male does not transmit the disorder to his sons.
- **Pattern of inheritance:** **Transmission is through female carrier** (heterozygous).

TABLE 8.3: Examples of X-linked recessive disorders**Q. Name sex-linked inherited disorders.**

System affected	Examples
Blood	<ul style="list-style-type: none"> Hemophilia A and B Glucose-6-phosphate dehydrogenase deficiency Chronic granulomatous disease
Musculoskeletal system	<ul style="list-style-type: none"> Duchenne muscular dystrophy
Nervous system	<ul style="list-style-type: none"> Fragile-X syndrome
Metabolic disorders	<ul style="list-style-type: none"> Diabetes insipidus Lesch-Nyhan syndrome
Immune systems	<ul style="list-style-type: none"> Agammaglobulinemia Wiskott-Aldrich syndrome

- **Risks of transmission to children (offspring):**
 - An affected male does not transmit the disorder to his sons, but all daughters are carriers.
 - Sons of heterozygous women have 50% chance of receiving the mutant gene.

Examples of X-linked recessive disorders are shown in Table 8.3.

X-linked Dominant Disorders

General Features

They are very rare, e.g. vitamin D resistance rickets.

- **Location of mutant gene:** It is on the X chromosome and there is no transmission from affected male to son.
- **Required number of defective gene:** One copy of mutant gene is required for its effect.
 - Often lethal in males and so may be transmitted only in the female line.
 - Often lethal in affected males and they have affected mothers.
 - No carrier state.
 - More frequent in females than in males.
- **Risks of transmission to children (offspring):**
 - Transmitted by an affected heterozygous female to 50% of her sons and half her daughters
 - Transmitted by an affected male parent to all his daughters but none of his sons, if the female parent is unaffected.

X-linked dominant inheritance: Female carriers are asymptomatic.

DEVELOPMENTAL DEFECTS

Developmental defects are a group of abnormalities that occur during fetal life due to errors in morphogenesis.

Definitions

Q. Write short note on malformations, disruption and deformities with examples.

- **Congenital anomaly (birth defect/congenital defect/congenital disorder):** The term congenital means “born with”. All types of the **structural abnormalities** or defects that are present at birth are termed as **congenital anomalies**.
- A **malformation** is a primary (or intrinsic) **structural defect** occurring during the development of an organ or tissue. It may be due to a single gene or chromosomal defect, but are more commonly multifactorial in origin. Malformations may involve one organ /system or multiple systems.
 - **Single system defect** (single abnormality): Single abnormalities may have a genetic or non-genetic basis. Examples, congenital heart defect (such as ventricular or atrial septal defects), anencephaly (absence of the brain), cleft lip and/or palate and neural tube defects.
 - **Multiple malformation syndromes (multiple abnormalities):** It consists of defects in two or more systems and is more likely to be due to chromosomal abnormalities.
 - **Syndrome:** When a **combination of congenital abnormalities** occur together repeatedly in a consistent pattern due to a single underlying cause, it is termed as “syndrome”.
- **Dysmorphology** is the study of **malformations arising from abnormal embryogenesis**.
- **Agenesis** is the complete absence of an organ, e.g. unilateral or bilateral agenesis of kidney.
- **Aplasia** is the absence of development of an organ, e.g. aplasia of lung.
- **Hypoplasia** is incomplete development of an organ which does not reach the normal adult size, e.g. microglossia.
- **Atresia** refers to incomplete formation of lumen in hollow viscus, e.g. esophageal atresia.

LYON HYPOTHESIS

Q. Write short note on Lyon hypothesis.

In 1961, Lyon outlined the idea of X-inactivation, now known as the Lyon hypothesis. It states that **only one of the X chromosomes is genetically active** and:

1. **Other X of either maternal or paternal origin is inactivated** during early stage of embryonic development.
2. **Inactivation of either the maternal or paternal X** occurs at random among all the cells during about 16th day of embryonic life.
3. **Inactivation of the same X chromosome persists in all the cells derived from each precursor cell.**

DEMONSTRATION OF SEX CHROMATIN

Q. Write short note on Barr body and sex chromatin.

There are two simple methods:

1. Buccal smear for Barr body (sex chromatin)
2. Leukocytes—nuclear sexing.

Barr body: Attached to inner aspect of nuclear membrane and represents inactivated X-chromosome.

Genetic sex is determined by: Y chromosome.

Buccal Smear for Barr Body (Fig. 8.6A)

Number of Barr bodies = number of X-chromosomes–1.

- Barr and Bertram in 1949 identified the presence of **tiny dark granule adjacent to the nuclear membrane**. This granule is known as the **Barr body, or X chromatin or sex chromatin**. It represents one inactive and condensed X chromosome in a female. The inactive X can be seen in the interphase nucleus as a darkly staining small mass in contact with the nuclear membrane.
- Barr body:
 - Absent in normal males
 - Normal female has 1 Barr body.
- The **number of Barr bodies in a cell depends upon the number of X chromosome**. It is always one less than the number of X chromosomes. Thus, normal cells in female (XX) have one Barr body and presence of Barr bodies indicates female genotype. Normal cells in male (XY) have no Barr bodies because they have only one active X chromosome. The XXXY cells have two Barr bodies.
- **Demonstration:** Buccal smears are used for demonstration of Barr body are prepared with a thin wooden spatula, by scraping the buccal mucosa. Smears are stained by Papanicolaou stain.

Leukocytes—Nuclear Sexing (Fig. 8.6B)

- Neutrophils in the peripheral smear may also be examined for nuclear sexing. Abnormalities of sex chromosomes can be diagnosed by nuclear sexing. In a normal female (XX), the **neutrophils in a peripheral smear show a drumstick** which is counterpart of Barr body in buccal smear.
- **Absence of drumstick** is observed in **Turner syndrome (XO)**, while **one drumstick** is found in males with **Klinefelter syndrome (XXY)**.

Y chromosome: Irrespective of the number of X chromosomes, the presence of a single Y determines the male sex.

CYTOGENETICS

Cytogenetics is a branch of genetics that deals with the **study of the chromosomes**. Karyotype is one of the basic tools of cytogenetics.

Techniques of Cytogenetics

It can be broadly divided into:

- **Conventional cytogenetics:** It is the routine chromosome analysis.
- **Molecular cytogenetics:** Molecular genetics (often called as 'DNA technology') is the study of the genetic material at the level of the individual nucleotide bases of DNA.

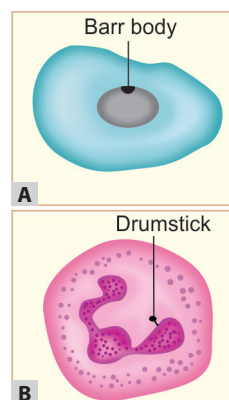
Karyotyping

Q. Write short essay/note on karyotyping.

Karyotype : Standard arrangement of photographed or image of chromosomes in metaphase arranged in order of decreasing length.

Karyotyping detects:

1. Chromosomal abnormalities—abnormal number (aneuploidy)
 2. Large deletions
 3. Translocations
 4. Unknown mutations.
- The **chromosomal constitution of a cell or individual** is known as the **karyotype**. The normal human karyotypes contain 22 pairs of autosomal chromosomes and one pair of sex chromosomes. Normal karyotype for females is denoted as 46, XX and for males as 46, XY.



Turner syndrome: No Barr body

Klinefelter syndrome: Male with Barr body

Figs 8.6A and B: (A) Nuclei of intermediate squamous cell with Barr body; (B) Neutrophil with drumstick

- **Study of structural patterns** of the chromosomes in a sample of cells is known as **karyotyping**. This includes both the **number and appearance (photomicrograph) of complete set of chromosomes**. Karyotyping requires cells to be in a state of division and arresting this cell division at the metaphase of cell cycle.

Source of chromosome: To produce karyotype, it is necessary to obtain cells capable of growth and division. Cells for chromosomal study may be obtained from either by culture or directly.

- **Culture:** The source may be fibroblast or cells obtained by amniocentesis (amniotic fluid) or peripheral blood. The more commonly used cell for chromosomal study is **circulating lymphocyte** obtained from the blood sample cultured in a media.
- **Direct:** Cells obtained from **bone marrow and chorion villous** biopsy samples may be used without culture.

Staining: There are many staining methods using **specific dyes** to identify individual chromosomes. Most commonly used is **Giemsa stain**.

Disadvantages of karyotyping (conventional cytogenetics)

1. Cannot detect minor (subtle/submicroscopic) deletions/mutations
2. Cannot identify gene amplifications
3. Metaphasic arrest is difficult in solid tumors.

Classification of Chromosomes in Karyotyping

There are various systems used for study the morphology of the chromosomes.

Denver system of classification: In this system, the chromosomes are grouped from A to G according to the length and position of the centromere of the chromosomes.

Paris system of classification: This is a universally accepted classification. According to this, the chromosomes are identified based on the various banding patterns.

Chromosomal Banding

Banding is a method to **study the structure of a chromosome**. In this method, chromosomes are stained by a special stain (e.g. Giemsa) which binds to specific bands of chromosome. Each chromosome shows a characteristic banding pattern (light and dark bands) which will help to identify them.

Techniques: Different banding techniques are:

- **G-banding** (G for Giemsa): It is most commonly used and shows a series of light and dark stained bands (Fig. 8.7). Giemsa stain is specific for the phosphate groups of DNA.
- **Q-banding** (Quinacrine fluorescent stain).
- **R-banding:** It is the reverse of G-banding (the R stands for “reverse”).

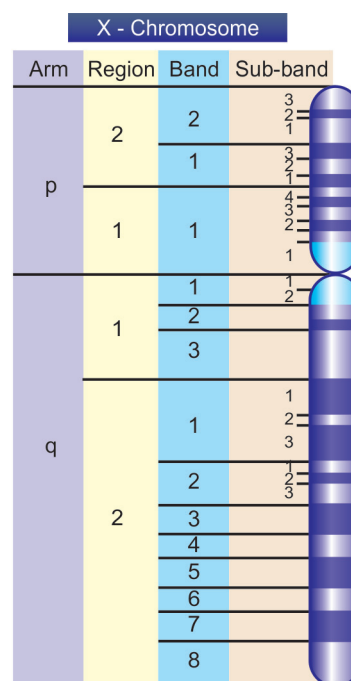


Fig. 8.7: G-banded karyotype shows the banding pattern of the X-chromosome with nomenclature of arms, regions, bands, and sub-bands

- **C-banding** (centromeric): This method stains centromeres.
- **T-banding:** It stains the terminal ends of chromosomes (telomeres).
- **High resolution banding:** It provides greater sensitivity.

Routine technique for karyotyping using light microscopy is: G-banding.

G-banding: Karyotyping most commonly done under light microscopy.

Karyotype Analysis

Long and short arm of chromosome are called respectively: q and p.

Karyotypes are usually described using a standard short hand format in the following order:

- **Total number of chromosomes.**
- **Sex chromosome** constitution
- **Description of abnormalities** in ascending numerical order.
 - **Short arm or long arm:** The short arm of chromosome is designated “p” (petit) and the long arm “q” (queue).
 - **Region:** Each arm of the chromosome is divided into two or more regions. The regions are numbered (e.g. 1, 2, 3) from the centromere outward.

- ◆ Bands and sub-bands: Each region is further subdivided into bands and sub-bands, and these are ordered numerically as well. This will help for precise localization of the gene.
- **Structural changes in chromosomes**

Example: The notation *Xp21.2* refers to a chromosomal segment located on the short arm of the X chromosome, in region 2, band 1, and sub-band 2.

Karyotype: Size, shape and number of chromosome.

Uses of Karyotyping

- **For diagnosis:** Diagnosis of genetic disorders including prenatal diagnosis.
- **To detect the cause of repeated abortions:** Many chromosomal aberrations can cause repeated spontaneous abortions and they can be identified by karyotyping.
- **Prognostic value:** Identification of specific chromosomal anomalies in certain cancers will help in predicting the course and prognosis (e.g. Philadelphia chromosome in chronic myeloid leukemia).

CHROMOSOMAL ABERRATIONS

Classification (Refer Box 8.1)

1. Numerical chromosomal aberrations
2. Structural chromosomal aberrations

Both may involve either the autosomes or the sex chromosomes.

Numerical Chromosomal Aberrations

Total number of chromosomes may be either increased or decreased. The deviation from the normal number of chromosomes is called as numerical chromosomal aberrations.

Types of Numerical Aberrations

- a. **Aneuploidy:** It is defined as a **chromosome number that is not a multiple of 23** (the normal haploid number-*n*).
 - **Trisomy:** It is **numerical abnormality with the presence of one extra chromosome** ($2n + 1$). It may involve either sex chromosomes or autosomes. For example, **Down's syndrome** (trisomy 21) have three copies of chromosome 21 (47 XX, +21).
 - **Monosomy:** It is the **numerical abnormality with the absence or loss of one chromosome** ($2n - 1$). It may involve autosomes or sex chromosomes. For example, **Turner syndrome** 45 XO instead of normal 46 XX.

- b. **Polyploidy:** This term used when the **chromosome number is a multiple greater than two of the haploid number** (multiples of haploid number 23).
- c. **Mosaicism:** It is the presence of **two or more populations of cells with different chromosomal complement in an individual**.

Autosomal monosomy: Not compatible with life.

Nondysjunction: Unequal separation of chromosomes during meiosis.

Mosaicism: Nondysjunction during mitosis.

Structural Chromosomal Aberration

Aberration of structure of one or more chromosomes may **occur during either mitosis or meiosis**. The various types (Table 8.1) include:

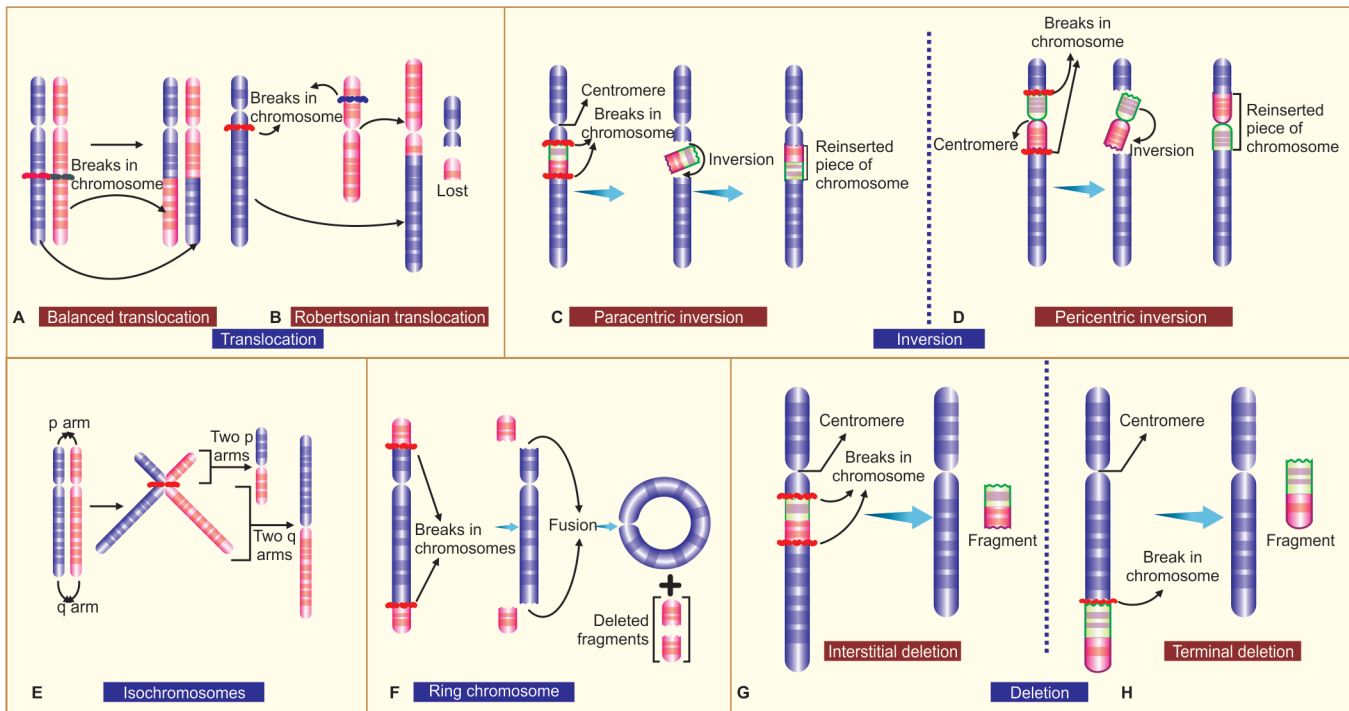
Translocations: It is a structural alteration between two chromosomes in which **segment of one chromosome gets detached and is transferred to another chromosome**. It can be:

- **Balanced reciprocal translocations** (Fig. 8.8A): It is characterized by single breaks in each of two chromosomes with exchange of genetic material distal to the break.
- **Robertsonian translocation/centric fusion** (Fig. 8.8B): It is a translocation between two acrocentric chromosomes. The breaks occur close to the centromeres of each chromosome. Transfer of the segments leads to one very large chromosome and one extremely small one. The small one is because of fusion of short arms of both chromosomes which lack a centromere and is lost in subsequent divisions. This loss is compatible with life but it may produce abnormal progeny.

Inversion: It involves two breaks within a single chromosome, the affected segment inverts with reattachment of the inverted segment. The genetic material is transferred within the same chromosome. Inversions are usually fully compatible with normal development. Two types of inversions are:

- **Paracentric inversions** (Fig. 8.8C) result from breaks on the same arm (either the short arm or the long arm) of the chromosome.
- **Pericentric inversions** (Fig. 8.8D) result from breaks on the opposite sides of the centromere where both the short and long arms are involved.

Isochromosome (Fig. 8.8E): They are formed due to faulty centromere division. Normally, centromeres divide in a plane parallel to long axis of the chromosome. If a centromere divides in a plane transverse to the long axis, it results in pair of isochromosomes. One pair consists of two short arms and the other of two long arms.



Figs 8.8A to H: Types of chromosomal rearrangements

Ring chromosome (Fig. 8.8F): It is a special form of deletion. Ring chromosomes are formed by a break at both the ends of a chromosome with fusion of the damaged ends. The consequences depend on the amount of genetic material lost due to the break. Loss of significant amount of genetic material will result in phenotypic abnormalities. It is expressed as $46,XY,r(14)$. Ring chromosomes do not behave normally in meiosis or mitosis and usually result in serious consequences.

Deletion (Figs 8.2G and H): It is the loss of a part of a chromosome. It is of two types namely: interstitial (middle) and terminal (rare).

Insertion: It is a form of nonreciprocal translocation in which a fragment of chromosome is transferred and inserted into a nonhomologous chromosome. Two breaks occur in one chromosome, which releases a chromosomal fragment. This fragment is inserted into another chromosome following one break in the receiving chromosome, to insert this fragment.

Acrocentric transmission is called: Robertsonian translocation.

Structural chromosomal aberrations:

- Translocation
- Inversion
- Isochromosome
- Ring chromosome
- Deletion
- Insertion.

GENOMIC IMPRINTING

All individuals inherit two copies of each autosomal gene. One of these is from maternal and other is from paternal chromosomes. It was earlier thought that there is no functional difference between the alleles derived from the mother or the father. It is found that different clinical features can result, depending on whether a gene is inherited from the father or mother. These differences are due to an epigenetic process, called **imprinting**. Mostly imprinting selectively inactivates either the maternal or paternal allele. Thus, in **maternal imprinting** there is silencing of the maternal allele, whereas in **paternal imprinting** there is inactivation of paternal allele. Imprinting occurs during gametogenesis in the ovum or the sperm, before fertilization, and then is stably transmitted to all somatic cells through mitosis. The pattern of imprinting is maintained to variable degrees in different tissues.

MOLECULAR GENETIC DIAGNOSIS

Diagnostic Methods and Indications for Genetic Testing

Q. Laboratory diagnosis of genetic diseases.

Genetic disease may be caused from single base substitutions up to gains or losses of entire chromosomes. These can be detected by various genetic tests.

Timing of Genetic Tests

Depending on the timing of performing, these genetic tests can be divided into four types.

1. **Preimplantation testing:** Done before conception (i.e. when one or two of the parents are carriers of a certain trait) on embryos created in vitro prior to uterine implantation to detect genetic changes in embryos. This is performed when parents known to be at risk of having a child with a genetic disorder. It eliminates the chance of generational transmission of a familial disease.
2. **Prenatal testing:** These are done after conception and its indications are listed in Box 8.2.
 - Genetic test is performed on cells obtained by amniocentesis, chorionic villus biopsy, or umbilical cord blood. About 10% of the free DNA in a pregnant mother's blood is of fetal origin, and new noninvasive prenatal diagnostics tests use this source of DNA.
3. **Newborn and children genetic testing:** It is used to identify genetic disorders just after birth, so that it can be treated early in life. Indications for newborn and child genetic analysis are shown in Box 8.3. It is usually performed on peripheral blood DNA.
4. **Genetic test in adults and older individuals**
Its indications are listed in Box 8.4.

BOX 8.2: Indications for prenatal testing

- A mother of advanced age (>35 years) who have increased risk of trisomies
- A parent to carry a balanced chromosomal rearrangement which increases the frequency of abnormal chromosome segregation during meiosis and the risk of aneuploidy in the fertilized ovum
- A fetus with abnormalities detected by ultrasound
- Routine maternal blood screening, indicating an increased risk of Down syndrome or another trisomy.

BOX 8.3: Indications for newborn and children genetic analysis

- Major /multiple congenital anomalies
- Suspicion of a metabolic syndrome (e.g. phenylketonuria)
- Unexplained mental retardation and/or developmental delay
- Suspected aneuploidy (e.g. features of Down syndrome) or other syndromic chromosomal abnormality (e.g. Turner syndrome)
- Suspected monogenic disease.

BOX 8.4: Indications for genetic test in adolescence and adulthood

- Inherited cancer syndromes (family history of cancer with a known or suspected inherited predisposition or an unusual cancer presentation)
- Atypically mild monogenic disease (e.g. attenuated cystic fibrosis)
- Family history of an adult-onset of neurodegenerative disorders (e.g. familial Alzheimer disease, Huntington disease).

Indications for Analysis of Acquired Genetic Alterations (Box 8.5)

BOX 8.5: Common indications for analysis of acquired genetic alterations

1. Diagnosis and management of cancer
 - To detect tumor-specific acquired mutations and cytogenetic alterations, e.g. BCR-ABL fusion genes in chronic myelogenous leukemia, or CML
 - To identify specific genetic alterations which helps in choosing therapy, e.g. HER2 (ERBB2 amplification in breast cancer or EGFR (ERBB1 mutations in lung cancer
 - To detect minimal residual disease, e.g. detection of BCR-ABL by PCR in CML
2. Diagnosis and management of infectious disease
 - To detect microorganism-specific genetic material for definitive diagnosis, e.g. HIV, mycobacteria
 - To identify specific genetic alterations in the genomes of microbes in case of drug resistance
 - To determine efficacy of treatment, e.g. to assess viral loads in HIV, hepatitis C virus infection.

Genetic Tests

Polymerase Chain Reaction (PCR)

It is widely used, powerful tool in the molecular diagnosis of human disease.

Principle: In PCR, the double-stranded DNA of interest is separated into two individual strands. Each strand is then allowed to hybridize with a primer. The specific fragment of DNA is amplified to generate large quantities (thousands to millions of copies) of particular DNA fragments of interest.

- Subsequent analysis can be done by different techniques such as (1) **Sanger sequencing**, (2) **pyrosequencing**, (3) **single-base primer extension**, (4) **restriction fragment length analysis**, (5) **amplicon length analysis** and (6) **real-time PCR**.

Advantages

- **Wide range of samples:** PCR allows analysis of DNA from any cellular source containing nuclei.
- **Small quantity required:** PCR needs very small quantity of genetic material and can amplify DNA from even single cell.
- **Sensitivity:** It has remarkable sensitivity.
- **Rapid:** It produces DNA fragments in a matter of hours.

Disadvantages

- It requires knowledge of the nucleotide sequence of the target DNA fragment.
- It can amplify DNA fragments usually up to 1 kb.

Molecular Analysis of Genomic Alterations

Genetic lesions with large deletions, duplications, or more complex rearrangements cannot easily assayed by standard PCR methods. Such genomic alterations can be studied by hybridization-based techniques.

1. **Fluorescence in situ hybridization (FISH):** Uses DNA probes which detect and localize sequences specific to particular chromosomal regions.

Fluorescent in situ hybridization (FISH):

1. Identify known deletions irrespective of size
2. Identify translocation by different probes
3. Identify gene amplification
4. No need of metaphasic arrest.

Fluorescent in situ hybridization (FISH): Cannot detect unknown chromosomal changes

2. **Multiplex ligation-dependent probe amplification (MLPA):** Blends DNA hybridization, DNA ligation, and PCR amplification to detect deletions and duplications of genome of any size. It detects genetic alteration that are too large to be detected by PCR and too small to be identified by FISH. It can either be performed on dividing cells (metaphase chromosomes) or nondividing cells (interphase nuclei) making it much more versatile than traditional karyotyping.
3. **Southern blotting:** Detects changes in the structure of specific loci.
4. **Cytogenomic array technology:** It detects genomic abnormalities without prior knowledge in contrast to FISH which needs prior knowledge of the one or few specific chromosomal regions suspected of being altered in the test sample.

Next-Generation Sequencing

- **Next-generation sequencing (NGS) consists of several newer DNA sequencing technologies which can**

produce large amounts of sequence data in a massively parallel manner.

- NGS can perform previously impossible analyses at extremely low relative cost.
- **Advantage:** Any DNA from almost any source can be used and are well suited to heterogeneous DNA samples. NGS is useful for detecting genetic anomalies of essentially any size scale ranging from SNPs to very large rearrangements including aneuploidy.

STORAGE DISEASES

Q. Name storage disorders.

Lysosomal storage disorders:

- Inherited
- Mutation in genes that code lysosomal hydrolases.
- Lysosomal enzymes are used for the intracellular digestion/ degradation of many complex biological macromolecules.
- **Deficiency of lysosomal enzymes:** Inherited deficiency of lysosomal enzyme may cause **incomplete catabolism of its normal macromolecular substrate**. This can lead to the **accumulation of the partially degraded insoluble substrate within the lysosomes**. The inherited disorders result from mutations in genes that encode lysosomal hydrolases are known as lysosomal storage disorders.

General Features

- Lysosomal disorders are **transmitted as autosomal recessive disorder**.
- Usually **detected in infants and young children**.
- Hepatosplenomegaly due to accumulation of insoluble intermediate compounds in the mononuclear phagocytes.
- CNS involvement is associated with damage to neurons.

Classification of lysosomal storage disorders: They are classified according to the biochemical nature of the metabolite accumulated within the lysosomes. The subgroups include **glycogenoses, sphingolipidoses (lipidoses), sulfatidoses, and mucopolysaccharidoses (MPSS)**.

Niemann-Pick Disease

Q. Write short note on Niemann-Pick disease and its enzyme deficiency.

Niemann-Pick disease (NPD) :

- Lysosomal storage disorders (lipidoses)
- Inherited deficiency of sphingomyelinase
- Lysosomal accumulation of sphingomyelin.

Niemann-Pick disease (NPD) is one of the **lysosomal storage disorders (lipidoses)** that are characterized by lysosomal accumulation of **sphingomyelin** due to an **inherited deficiency of sphingomyelinase**.

- Mode of transmission: **Autosomal recessive**.

Classification of Niemann-Pick Disease

- **Type A:** It is a **severe infantile form** with **almost complete deficiency of sphingomyelinase**. It is characterized by **extensive neurologic involvement, massive visceromegaly**, marked accumulations of **sphingomyelin in liver and spleen**, and progressive wasting and death occurring by 3 years of age.
- **Type B:** It usually presents with **hepatosplenomegaly** and generally **without involvement of central nervous system**. They usually survive into adulthood.
- **Type C:** It is **more common than types A and B**. It is due to **mutations in two genes** namely, **NPC1 and NPC2**. It is due to **primary defect in lipid transport**. Commonly manifests in childhood with ataxia, vertical supranuclear gaze palsy, dysarthria, dystonia, and psychomotor regression.

MORPHOLOGY

Deficiency of sphingomyelinase enzyme blocks degradation of the lipid—sphingomyelin → accumulates inside the lysosomes of cells of the mononuclear phagocyte system.

- **Organs involved:** They show **moderate to marked enlargement**.
 - **Brain:** It shows shrunken gyri and widened sulci. Microscopically, the **neurons show vacuolation and ballooning**, which in time leads to cell death and loss of brain substance.
 - **Retina:** It shows a **cherry-red spot**.
 - **Other organs:** **Spleen (massively enlarged)**, liver, lymph nodes, bone marrow, tonsils, gastrointestinal tract, and lungs.
- **Light microscopy:**
 - **Characteristic storage cell is a macrophage with many, small, uniform vacuoles** (contains sphingomyelin and cholesterol) within the cytoplasm.
 - These lipid-laden foam cells are large (20 to 90 μm in diameter) and frozen sections—vacuoles take up fat stains.
- **Electron microscopy:** The **lipid vacuole resembles concentric lamellated myelin figures** which are called “zebra” bodies.

Niemann-Pick disease type A and B:

- Diagnosis and detection of carriers by estimation of sphingomyelinase activity in leukocytes/cultured fibroblasts.
- Antenatal diagnosis by enzyme assay or DNA probe analysis.

NPD type 3:

- More common than type A and B
- Mutations in NPC1 and NPC2 gene.

Tay-Sachs Disease (GM₂ Gangliosidosis: Hexosaminidase β-Subunit Deficiency)

Tay-Sachs disease is a GM₂ gangliosidosis caused by deficiency of enzyme hexosaminidase, subunit.

- Tay-Sachs disease is inherited as an **autosomal recessive** trait.
- **Most common** form of GM₂ gangliosidosis.
- Characterized genetic **mutations in HEXA** gene on chromosome 15 and a severe **deficiency of β-subunit hexosaminidase A** enzymes.
- **Hexosaminidase A** enzymes is **absent in almost all the tissues**.

MORPHOLOGY

- **GM₂ ganglioside** accumulates in many tissues such as CNS, retina, heart, liver and spleen.
- **Special stains:** Special stains for fat such as oil red O and Sudan black B stain positive with gangliosides.
- **Light microscopy:**
 - **Neurons:** **Ballooned with many cytoplasmic vacuoles**, each representing a severely distended lysosome filled with gangliosides → followed by destruction of neurons, proliferation of microglia, and accumulation of lipids in phagocytes within the brain substance.
 - **Retina:** Ganglion cells in the retina distended with GM₂ ganglioside, more prominent at the margins of the macula → gives rise to characteristic **cherry-red spot** in the macula. Cherry-red spot is also seen in other storage disorders affecting the neurons.
- **Electron microscopy:** Most prominent features is **prominent lysosomes with whorled configurations** which represents onion-skin layers of membranes.

Clinical Features

- Usually presents between 6 and 10 months of age.
- Clinical features are mainly due to **neuronal involvement in the central and autonomic nervous systems and retina**. Symptoms include progressive motor and mental deterioration, blindness, and increasing dementia.
- Ophthalmoscopy **cherry-red spot** in the macula.
- Over the span of 1 or 2 years a complete vegetative state is reached. Most children die before 3 years of age.
- **Antenatal diagnosis and carrier detection:** It can be done by enzyme assays and DNA-based analysis.

Gaucher Disease

Q. Write short essay/note on Gaucher disease.

- **Most common lysosomal storage disorder** due to mutation in the gene that encodes glucocerebrosidase (cleaves glucose residue from ceramide).

- **Autosomal recessive mode of transmission.**
- Due to **deficiency of enzyme glucocerebrosidase** → results in **accumulation of glucocerebroside**, mainly in lysosomes of macrophage.
- **Pathological changes** are both due to:
 - **Accumulation of glucocerebroside**
 - **Activation of macrophages** → secretes cytokines such as IL-1, IL-6, and tumor necrosis factor (TNF).

Gaucher disease:

- Autosomal recessive
- Deficiency of enzyme glucocerebrosidase
- Accumulation of glucocerebroside, mainly in lysosomes of macrophage.

Clinical Subtypes

Q. Write short note on enzyme deficiency in Gaucher disease.

There are three variants namely:

- **Type I or the chronic non-neuronopathic form:**
 - **Most common type** (about 99% of cases)
 - **Glucocerebrosides** are stored only in the **mononuclear phagocytes throughout the body** mainly in the **spleen and skeletal system**. It does not involve the brain.
- **Type II or acute neuronopathic Gaucher disease:**
 - **Infantile acute cerebral pattern**
 - Almost **complete absence of glucocerebrosidase** activity in the tissues → **progressive involvement of CNS** → **death at an early age**.
- **Type III:** It is **intermediate between types I and II**.

MORPHOLOGY

Q. Write short note on Gaucher cell and its morphology.

Light microscopy: Gaucher cells are hallmark of this disorder and its characteristics are:

- **Enlarged, phagocytic cells** (sometimes up to 100 µm in diameter) distended with **massive amount of glucocerebrosides**.
- **Seen throughout the body in virtually all organs**, especially in the **spleen, liver, bone marrow**, lymph nodes, tonsils, thymus, and Peyer's patches.
- Gaucher cells have a **fibrillary type of cytoplasm** like a **crumpled/wrinkled tissue paper** (Fig. 8.9) and one or more dark, eccentrically placed nuclei.
- The **cytoplasm** of Gaucher cells stain intensely **positive with Periodic acid–Schiff**.

Electron microscopy: The fibrillary cytoplasm appears as elongated, distended lysosomes, containing the stored lipid arranged in parallel layers of tubular structures.

Gaucher cell: Fibrillary type of cytoplasm like a crumpled tissue paper.

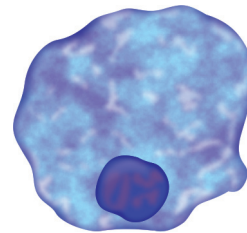


Fig. 8.9: Appearance of Gaucher cells

Clinical Features

Type I

- Manifests in adult life and follows a progressive course.
- **Spleen is enlarged, sometimes up to 10 kg and hypersplenism** may lead to pancytopenia or thrombocytopenia. Hepatomegaly is also seen.
- **Bone marrow:** Accumulation of Gaucher cells produces extensive expansion of the marrow space, bone erosion, focal lytic bone lesions, osteonecrosis, osteopenia, and pathologic fractures.

Types II and III

- In patients with CNS involvement, it may produce cerebral dysfunction, convulsions, and progressive mental deterioration. Gaucher cells are seen in the Virchow-Robin spaces.

TRISOMY 21 (DOWN SYNDROME)

Q. Write short essay/note on Down syndrome.

- Down syndrome was first described by Dr John Langdon Down.
- It is a **cytogenetic disorder involving autosome**.
- **Most common chromosomal disorder** and is a **leading cause of mental retardation**.
- About **95% of these individuals have trisomy 21** (extra copy of chromosome 21), resulting in **chromosome count of 47** instead of normal 46.
- Parents of children with Down syndrome are normal and have a normal karyotype.

Etiology and Pathogenesis

- **Maternal age:** **Older mothers** (above 45 years of age) have much **greater risk**.
- **Other factors:** Increased incidence may be associated with exposure of mother to pesticides, electromagnetic fields, anesthetic drugs, alcohol and caffeine.

Mechanism of trisomy 21: The **three copies of chromosome 21** in somatic cells cause Down syndrome. It may be due to:

- **Nondisjunction in the first meiotic division of gametogenesis** and is responsible for trisomy 21 in most (95%) of the patients.
- Robertsonian translocation in about 5% of cases.
- Mosaicism in about 1% of cases.

Down syndrome: Non-disjunction of chromosome 21.

Down syndrome: Most common cause is maternal meiotic non-dysfunction.

Down syndrome: Caused by Robertsonian translocation and Mosaicism has no relation with maternal age.

Down syndrome has extra copy of chromosome 21:

- Trisomy 21
- Mosaic 21
- Robertsonian translocation (14,21).

Chromosomal abnormality in mongolism (obsolete term) is: Trisomy 21.

Clinical Features

Down syndrome: Most common cause of mental retardation.

Diagnosis of Down syndrome is **usually apparent at the time of birth** by the infant's **characteristic craniofacial appearance** (Fig. 8.10). The **diagnosis is confirmed by cytogenetic analysis**.

Characteristic features appear as the child grows.

- **Mental status:** Children are **mentally retarded with low IQ** (25–50).
- **Craniofacial features:** Diagnostic clinical features are:
 - **Flat face and occiput, with a low-bridged nose, reduced interpupillary distance and oblique palpebral fissures.**
 - Epicanthal folds of the **eyes impart an oriental appearance** (obsolete term mongolism).
 - Speckled appearance of the iris (**Brushfield spots**).
 - **Enlarged and malformed ears.**
 - A prominent tongue (**macroglossia**), which typically **lacks a central fissure** and protrudes through an open mouth.
- **Heart:** **Congenital cardiac anomalies** are responsible for the majority of the deaths in infancy and early childhood. The cardiac defects are:
 - **Septal and AV defect:** These defects may involve atrial septum (atrial septal defect), ventricular septum (ventricular septal defect), and one or more atrioventricular (AV) valves.

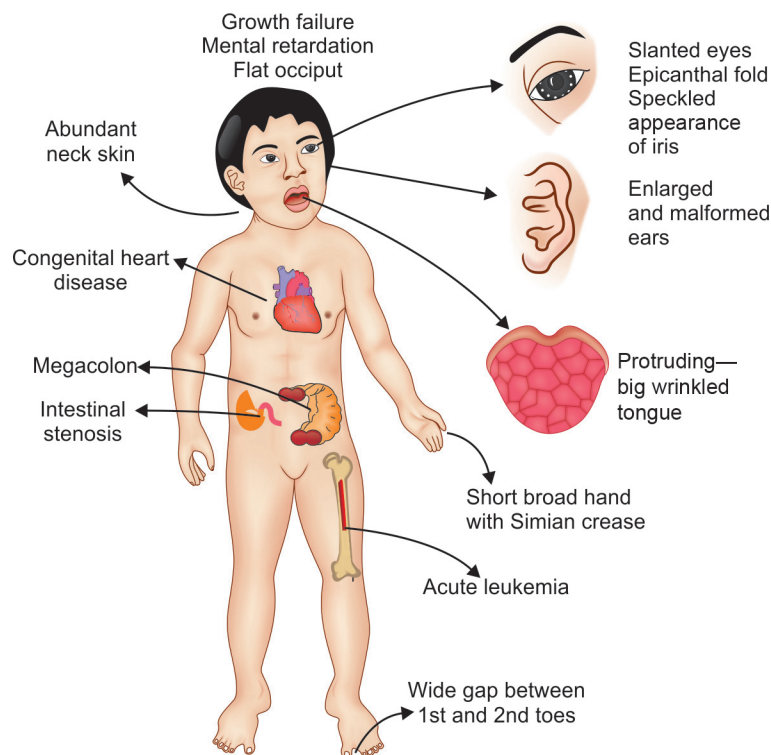


Fig. 8.10: Clinical features of Down syndrome

- Other cardiac anomalies: Tetralogy of Fallot and Patent ductus arteriosus.
- **Skeleton:** These **children are small** because of shorter bones of the ribs, pelvis, and extremities. The hands are broad and short and show a **Simian crease** (a single transverse crease across the palm). The fifth finger curves inwards.
- **Gastrointestinal tract:** It may show **esophageal/duodenal stenosis or atresia, imperforate anus and Hirschsprung disease** (megacolon).
- **Reproductive system:** **Men are sterile** because of spermatogenesis arrest.
- **Immune system:** Affected children are susceptible to serious infections due to defective immunity.
- **Endocrine system:** **Antithyroid antibodies** may cause hypothyroidism.
- **Hematologic disorders:** They have increased **risk of** both acute lymphoblastic and acute myeloid **leukemia**. The latter is most commonly acute megakaryoblastic leukemia.
- **Atlantoaxial instability:** It is characterized by excessive movement at the junction of the atlas (C1) and axis (C2) vertebrae, due to laxity of either bone or ligament. Neurological symptoms develop when spinal cord is compressed. Clinically, it may present with easy fatigability, difficulty in walking, abnormal gait, restricted neck mobility, torticollis, etc.

Down syndrome: Alzheimer disease at younger age.

KLINFELTER SYNDROME

Q. Write short essay/note on Klinefelter syndrome.

It is a cytogenetic disorder involving sex chromosomes.

Definition: Klinefelter syndrome (testicular dysgenesis) is characterized by **two or more X-chromosomes and one or more Y chromosomes**. It is an **important and most frequent genetic cause of male hypogonadism**.

It is the most important genetic disease involving **trisomy of sex chromosomes**; it is associated with **reduced spermatogenesis and male infertility**.

Pathogenesis

- Most of the patients with Klinefelter syndrome have an **extra X-chromosome (47 XXY karyotype)**. This complement of chromosomes results from **nondisjunction during the meiotic divisions in one of the parents**.
- A **minority of them are mosaic** (e.g. 46XY/47XXY) or have more than two X-chromosomes (e.g. 47,XXY/48,XXXY) and one or more Y-chromosomes.

- Regardless of the number of extra X-chromosomes (even up to 4), the Y-chromosome results in a male phenotype.

Klinefelter's syndrome: Two or more extra copy of X chromosomes and 1 Y chromosome.

Classic karyotype of Klinefelter's syndrome is: 47 XXY.

Clinical Features

Klinefelter syndrome: An important genetic cause of male infertility.

Klinefelter syndrome (Fig. 8.11) is usually diagnosed after puberty and **hypogonadism is a consistent finding**.

- Most of the patients are **tall and thin** with relatively long legs (eunuchoid body habitus).
- Mental retardation is uncommon, although average IQ is reduced.
- At puberty, **testes and penis remain small with lack of secondary male characteristics**.
- Female characteristics include a **high-pitched/deep voice, gynecomastia, and a female pattern of pubic hair**.
- **Hypogonadism, reduced levels of testosterone, remarkably high levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH)**.
- Reduced spermatogenesis → azoospermia → **infertility**. The testis may show atrophy of seminiferous tubules

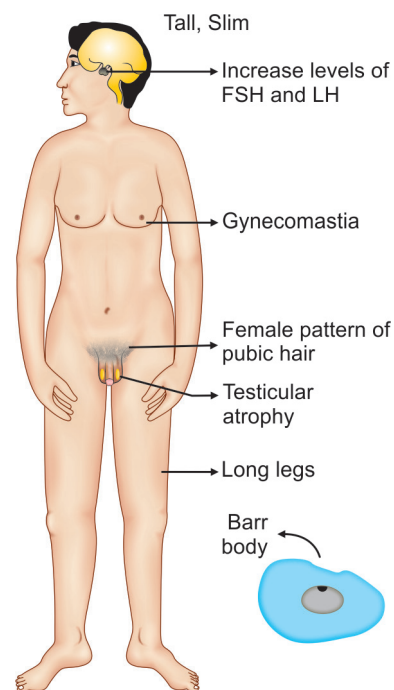


Fig. 8.11: Features of Klinefelter syndrome

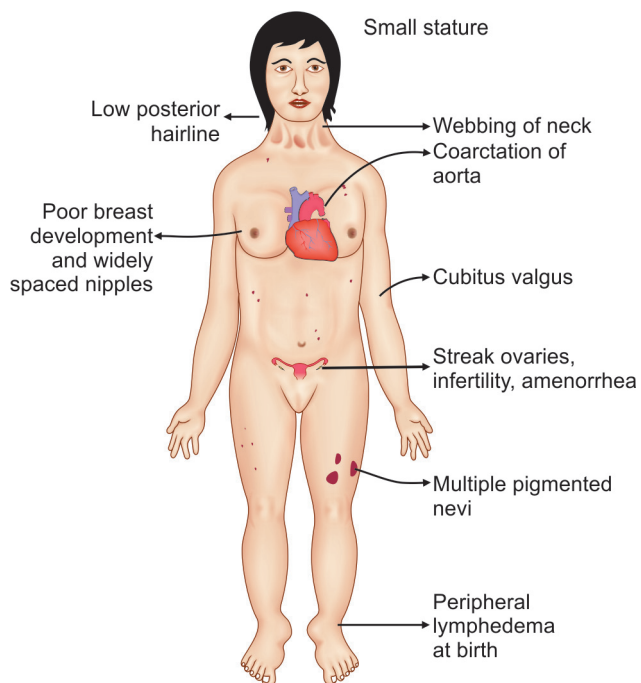


Fig. 8.12: Clinical features of Turner syndrome

containing pink, hyaline, collagenous ghosts. Leydig cells may appear prominent.

- **Increased incidence of type 2 diabetes and the metabolic syndrome.** Mitral valve prolapse is seen in about 50% of cases.
- **Higher risk for breast cancer, extragonadal germ cell tumors and autoimmune diseases** such as systemic lupus erythematosus.

Klinefelter syndrome: Increased levels of LH and FSH and decreased levels of testosterone.

TURNER SYNDROME

Q. Write short essay/note on Turner syndrome.

It is a cytogenetic disorder involving sex chromosomes.

TABLE 8.5: List of syndromes, associated genes and location of genes in the chromosomes

Syndrome	Gene	Location	Associated cancers and lesions
Hereditary nonpolyposis colonic cancer (HNPCC)	hMLH1	3p21	Colorectal carcinoma, endometrial cancer, transitional cell carcinoma of ureter and renal pelvis, carcinomas of stomach, small intestine, pancreas, ovary
	hMSH2	2p22.21	
	hMSH6	2p16	
	hPMS1	2q31.1	
	hPMS2	7p22.2	
von-Hippel-Lindau (VHL) syndrome	VHL	3p25	RCC, hemangioblastoma of CNS, pheochromocytoma

Contd...

Turner syndrome (Fig. 8.12) is a spectrum of abnormalities that **results from complete or partial monosomy of the X-chromosome in a phenotypic female**. It is characterized by hypogonadism and is the most common sex chromosome abnormality in females.

Turner syndrome: Primary amenorrhea (menopause before menarche).

Karyotypic Abnormalities

Three types of karyotypic abnormalities are found in Turner syndrome.

- **Missing of an entire X-chromosome:** It results in a 45 X karyotype.
- **Structural abnormalities of the X-chromosomes:** It include isochromosome of the long arm, translocations and deletions.
- **Mosaics:** 45 X cell population along with one or more karyotypically normal or abnormal cell types. Examples: (1) 45 X/46 XX; (2) 45 X/46 XY.

The molecular pathogenesis of Turner syndrome is not completely understood.

Barr body is not seen in: Turner syndrome.

Turner syndrome Karyotype: 45 X.

Clinical Features

Turner syndrome is usually **not discovered before puberty**. It presents with **failure to develop normal secondary sex characteristics**. Important diagnostic features are:

- **Adult women with short stature** (less than 5 ft tall), **primary amenorrhea and sterility**. At puberty, normal secondary sex characteristics fail to develop.
- **Webbed neck, low posterior hairline, wide carrying angle of the arms** (cubitus valgus), **broad chest** with **widely spaced nipples** and hyperconvex fingernails.

Contd...

Syndrome	Gene	Location	Associated cancers and lesions
Familial adenomatous polyposis (FAP)	APC	5q21	Adenocarcinoma of colon, extra-intestinal manifestations (congenital hypertrophy of retinal pigment epithelium)
Hereditary papillary RCC (renal cell carcinoma)	MET	7q31	Renal cell carcinoma
Tuberous sclerosis	TSC1	9 q34	Multiple hamartomas, RCC, astrocytoma
	TSC2	16p13	
Cowden's disease	PTEN	10q23.3	Cancer of breast, endometrium, thyroid
MEN-1	MEN-1	11q13	Pancreatic islet cell tumors, parathyroid hyperplasia, pituitary adenomas
MEN-2	RET	10q11.2	Medullary carcinoma of thyroid, pheochromocytoma, parathyroid hyperplasia
Wilms' tumor	WT	11p13	Wilms' tumor, aniridia, genitourinary abnormalities, mental retardation
Retinoblastoma	RB	13p14	Retinoblastoma, sarcomas (e.g. osteosarcoma), melanoma, malignant neoplasms of brain and meninges
Breast/ovarian syndrome	BRCA 1	17q21	Cancer of breast, ovary, colon, prostate
	BRCA2	13q12.3	Cancer of breast, ovary, colon, prostate, gallbladder, biliary tree, pancreas, stomach, melanoma
Neurofibromatosis-1	NF1	17q11	Neurofibroma, malignant peripheral nerve sheath tumor, acute myelogenous leukemia, brain tumors
Neurofibromatosis -2	NF2	22q12	Acoustic neuromas, meningiomas, gliomas, ependymomas
Li-Fraumeni	p53	17p13	Breast cancer, soft tissue sarcoma, osteosarcoma, brain tumors, adrenocortical carcinoma, Wilms' tumor, phyllodes tumor of breast, pancreatic cancers, leukemia, neuroblastoma
	hCHK2	22q12.1	
Peutz-Jegher's syndrome	STK11	19p13.3	Gastrointestinal carcinomas, carcinoma breast, testicular cancer, pancreatic cancer, benign pigmentation of skin and mucosa

- Other features: **Infantile genitalia, inadequate breast development, and little pubic hair.** The **ovaries** are converted to **fibrous streaks**.
- **Pigmented nevi** become prominent as the age advances.
- **Cardiovascular anomalies** like congenital heart disease particularly coarctation of the aorta.
- **Development of autoantibodies:** About 50% show autoantibodies that react with the thyroid gland → 50% of them may develop **hypothyroidism**.

Turner syndrome: Webbed neck, streak gonads, and menopause before menarche.

Turner syndrome: Most common genetic cause of primary amenorrhea.

Chromosomes involved in Patau syndrome: Chromosome 13.

List of syndromes, associated genes and location of genes in the chromosomes are presented in Table 8.5

Nutritional Disorders

COMMON VITAMIN DEFICIENCIES

Vitamins are vital organic substances, required in limited amounts, with key roles in certain metabolic pathways.

Categories

Thirteen vitamins are necessary for health and are categorized as follows:

- **Fat-soluble vitamins:** These include A, D, E and K. Fat-soluble vitamins are stored in the body, but their absorption may be poor in fat malabsorption disorders or in disturbances of digestive functions.
- **Water-soluble vitamins:** All other vitamins (vitamins of the B complex group and vitamin C).

FAT-SOLUBLE VITAMINS

Vitamin A (Retinol)

Vitamin A (retinol) is part of the family of retinoids which is present in food and the body as esters combined with long-chain fatty acids.

Functions

Vitamin A has several metabolic roles. The main functions of vitamin A in human are as follows:

- **Maintenance of normal vision:** It is one of the **major functions of vitamin A**. The visual process involves vitamin A-containing pigments.
- **Regulation of cell growth and differentiation:** It is one of the major functions of vitamin A. Retinol and retinoic acid are involved in the **control of proliferation and differentiation of epithelial cells**. Vitamin A and retinoids

play an important role in the orderly differentiation of mucus-secreting epithelium. In vitamin A deficiency, mucus-secreting cells are replaced by keratin-producing cells and this process is known as **squamous metaplasia**.

- **Regulation of lipid metabolism:** It is a key regulator of fatty acid metabolism, including fatty acid oxidation in fat tissue and muscle, adipogenesis and lipoprotein metabolism.
- **Host resistance to infections:**
 - **Immune function:** Vitamin A has ability to stimulate the immune system.
 - **Antioxidant:** Retinoids, β -carotene and some related carotenoids act as photoprotective and antioxidant agents.

Deficiency

Q. Write short essay/note on vitamin A deficiency.

Causes: Due to general undernutrition or as a secondary deficiency as a consequence of malabsorption of fats.

Pathologic Effects (Clinical Features) of Vitamin A Deficiency (Fig. 9.1)

- **Effects in the eye:**
 - **Night blindness:** Vitamin A is a component of rhodopsin and other visual pigments. Hence, one of the earliest manifestations of vitamin A deficiency is impaired vision, particularly impaired adaptation to the dark (*night blindness*).
 - **Xerophthalmia:** Vitamin A is necessary for maintaining the differentiation of epithelial cells. Persistent deficiency produces **epithelial metaplasia** and **keratinization**. In the eyes it produces keratinization of the cornea—**xerophthalmia** (dry eye). Initially, there

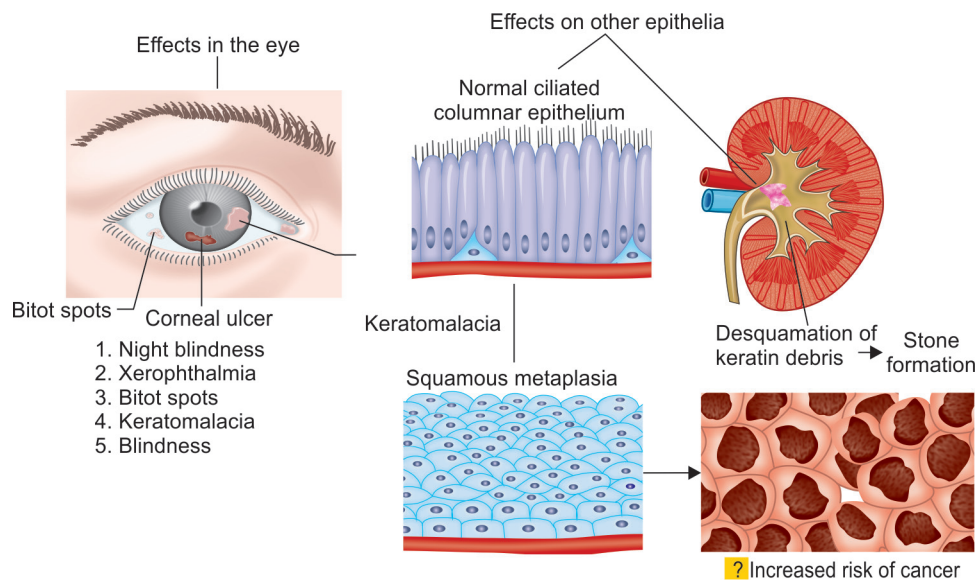


Fig. 9.1: Pathological effects of vitamin A deficiency

is dryness of the conjunctiva (**xerosis conjunctivae**) because of the replacement of the normal lacrimal and mucus-secreting epithelium by keratinized epithelium. Subsequently, there is a buildup of keratin debris in small opaque plaques which gives rise to characteristic **Bitot spots** that progresses to erosion of corneal surface, softening and destruction of the cornea (**keratomalacia**), scarring and **irreversible blindness**.

- **Effects on other epithelia:** The epithelium lining the upper respiratory passage and urinary tract also undergoes **squamous metaplasia**.
- **Immune deficiency:** It is responsible for higher mortality rates from common infections, such as measles, pneumonia and infectious diarrhea.
- Follicular hyperkeratosis.

Vitamin D

- Vitamin D is a **fat-soluble vitamin**.
- It is required for the maintenance of adequate plasma levels of calcium and phosphorus to support metabolic functions, bone mineralization and neuromuscular transmission.

Functions

1. **Regulation of plasma levels of calcium and phosphorus:** The main functions of vitamin D on calcium and phosphorus homeostasis are as follows:
 - **Stimulates intestinal absorption of calcium.**

- **Stimulates calcium reabsorption in the kidney.**
- **Interaction with PTH in the regulation of blood calcium.**
- **Mineralization of bone.**

2. Antiproliferative effects.

3. **Immunomodulatory:** Vitamin D is involved in the innate and adaptive immune system.

Deficiency

Causes

- Impaired cutaneous production due to **limited exposure to sunlight**.
- **Dietary absence:** Diets deficient in calcium and vitamin D.
- **Malabsorption.**

Milder forms of vitamin D deficiency is also called as **vitamin D insufficiency**, leads to an increased risk of bone loss and hip fractures in older adults.

Skeletal Effects of Vitamin D Deficiency

Q. Write short essay/note on rickets and its clinical features.

Rickets in Children (Fig. 9.2)

In children, before the closure of epiphyses, vitamin D deficiency causes retardation of growth associated with an expansion of the growth plate known as **rickets**. In the normal growth plate, there are three layers of chondrocytes namely (1) the reserve zone, (2) the proliferating zone and (3) the

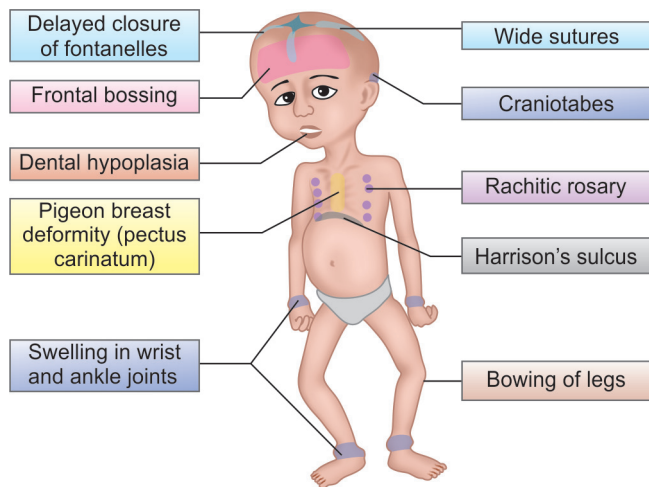


Fig. 9.2: Features of rickets

hypertrophic zone. Rickets due to impaired vitamin D action is characterized by expansion of the hypertrophic chondrocyte layer. In vitamin D deficiency, the hypophosphatemia due to secondary hyperparathyroidism is responsible for the development of the **rachitic growth plate**.

Gross skeletal changes in rickets: It depends on the severity and duration of the vitamin D deficiency and also the stresses to which individual bones are subjected.

During the nonambulatory stage of infancy:

1. Head

- **Craniotabes:** The head and chest are subjected to the greatest stresses. The softened occipital bones become flattened, and the parietal bones buckle inward by pressure; with the release of the pressure, elastic recoil snaps the bones back into their original positions (craniotabes). The **skull appears square and box-like**. Delayed closure of anterior fontanelle.
- **Frontal bossing:** Excess of osteoid produces **frontal bossing** and a squared appearance of the head.

Q. Write short note on rachitic rosary.

2. Chest

- **Rachitic rosary:** Overgrowth of cartilage or osteoid tissue at the costochondral junction causes deformation of the chest producing the “rachitic rosary.”
- **Pigeon breast/chest deformity:** The weakened metaphyseal areas of the ribs are subject to the pull of the respiratory muscles and thus, bend inward. This creates **anterior protrusion of the sternum** producing **pigeon breast deformity** (pectus carinatum).
- **Harrison's sulcus/groove:** It is due to indrawing of ribs on inspiration.

During the nonambulatory stage:

- **Lumbar lordosis:** This occurs when an ambulating child develops rickets. It is characterized by deformities affecting the spine, pelvis and tibia.
- **Bowing of the legs:** Due to affect on tibia.

Osteomalacia in Adults

Q. Write short note on osteomalacia.

Vitamin D deficiency in adults is accompanied by hypocalcemia and hypophosphatemia which result in **impaired** (hypo/under/inadequately) **mineralization of bone matrix** proteins, a condition known as osteomalacia. This hypomineralized bone matrix is biomechanically inferior (weak) to normal bone. This **bone is prone to bowing of weight-bearing extremities** and gross skeletal fractures or microfractures which are most likely to affect vertebral bodies and femoral necks.

Proximal Myopathy

It is observed both in children and in adults with severe vitamin D deficiency. It rapidly resolves by vitamin D treatment.

Hypocalcemic Tetany

Calcium is required for normal neural excitation and the relaxation of muscles. Hypocalcemic tetany is a **convulsive state** caused by an insufficient extracellular concentration of ionized calcium.

Nonskeletal Effects of Vitamin D

Vitamin D receptor is also present in various cells and tissues that are not involved in calcium and phosphorus homeostasis. Many cells, such as macrophages, keratinocytes, and tissues, such as breast, prostate and colon can produce 1,25-dihydroxyvitamin D.

- Low levels of 1,25-dihydroxyvitamin D (<20 ng/mL) may **increase in the incidence of cancers of colon, prostate and breast cancers**, but whether vitamin D supplement can reduce cancer risk has not known.

Vitamin C (Ascorbic Acid)

It is a **water-soluble vitamin**.

Functions

- **Hydroxylation of procollagen:** It is necessary for the formation of collagen from procollagen. It is involved in the hydroxylation of proline and lysine in procollagen to hydroxyproline and hydroxylysine in mature collagen.

- **Antioxidant properties:** Ascorbic acid is the most active powerful reducing agent controlling the redox potential within cells. Vitamin C can scavenge free radicals directly and can act indirectly by regenerating the antioxidant form of vitamin E.
- It is involved in intracellular electron transfer.
- **Promotion of nonheme iron absorption.**

Deficiency

Causes

- Ascorbic acid is present in abundance in many foods. Hence, its deficiency is rare.
- Rarely, it may occur as a secondary deficiency, particularly among older persons who live alone, and chronic alcoholics.

Effects of Deficiency (Fig. 9.3)

Q. Write short essay/ note on scurvy and its pathological findings.

Scurvy: It is characterized by:

- **Bone disease:** More common in growing children. It is characterized by **deranged formation of osteoid matrix**.
- **Hemorrhages:** Marked tendency to bleed into the skin (petechiae, ecchymoses, perifollicular hemorrhages), bleeding into muscles, joints and underneath peritoneum.
- **Delayed wound healing.**
- **Anemia.**
- **Gums: Inflamed and bleeding gums.**

Vitamin E

Vitamin E is a collective name for 8 stereoisomers of tocopherols and tocotrienols. The most important dietary form is α -tocopherol.

Functions

- **Antioxidant:** It prevents oxidation of low-density lipoproteins (LDLs) and polyunsaturated fatty acids in cell membranes by free radicals. Other antioxidants (e.g. vitamin C, glutathione) and enzymes maintain vitamin E in a reduced state. Acts in conjunction with other antioxidants, such as selenium.
- **It helps maintain cell membrane structure.**
- It affects DNA synthesis and cell signaling.
- **Anti-inflammatory:** Vitamin E also inhibits prostaglandin synthesis and the activities of protein kinase C and phospholipase A_2 .
- **Immune systems.**

Deficiency

- Dietary deficiency of vitamin E is very rare.
- Vitamin E deficiency is seen in only in premature infants and in severe and prolonged malabsorption diseases, such as celiac disease, or after small-intestinal resection.
- It can cause **mild hemolytic anemias, ataxia and visual scotomas.**

Vitamin K

Forms of vitamin K: There are two natural forms: Vitamin K₁ (phyloquinone) derived from vegetable (green leafy vegetables, such as kale and spinach) and animal sources (liver), and vitamin K₂ (menaquinone) which is synthesized by bacterial flora in the colon and in hepatic tissue. Phyloquinone can be converted to menaquinone in some organs.

Functions

- **Coagulation:** Vitamin K is a co-factor for carboxylation of glutamic acid which is necessary for the production of

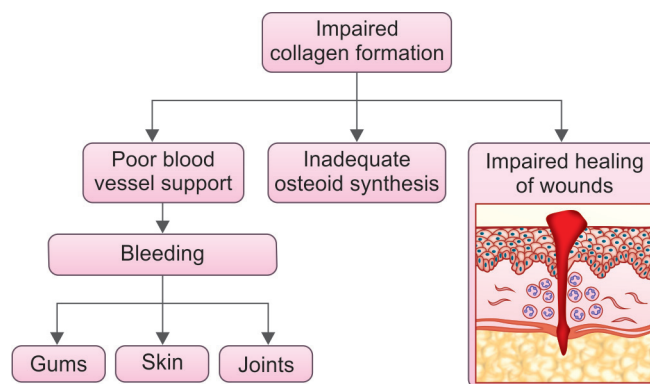


Fig. 9.3: Major consequences of vitamin C deficiency caused by impaired formation of collagen

carboxyglutamate (gla). Gla residues are found in four of the coagulation factor proteins (II, VII, IX and X). Thus, it is involved in coagulation process.

- **Others:** Other important gla proteins include osteocalcin (in bone) and matrix gla protein (vascular smooth muscle) that are important in **mineralization of bone**. However, the importance of vitamin K for mineralization of bone and prevention of vascular calcification is unknown.

Deficiency

Causes

- In adults:
 - **Chronic small-intestinal disease:** For example, celiac disease, Crohn's disease.
 - **Obstruction of biliary tracts:** In obstructive jaundice, dietary vitamin K is not absorbed and it is necessary to administer the vitamin in parenteral form before surgery.
 - After small-bowel resection.
 - **Broad-spectrum antibiotics:** They can precipitate vitamin K deficiency by reducing gut bacteria, which synthesize menaquinones, and by inhibiting the metabolism of vitamin K.
 - **Warfarin and related anticoagulants:** Warfarin-type drugs prevent the conversion of vitamin K to its active hydroquinone form.
- **Deficiency in newborn:** It is because of (1) low-fat stores, (2) low breast milk levels of vitamin K, (3) sterility of the infantile intestinal tract, (4) liver immaturity and (5) poor placental transport.

Effects of Deficiency

- Vitamin K deficiency leads to **delayed coagulation and bleeding**. Hence, the symptoms of vitamin K deficiency are due to hemorrhage.
- **Newborn:** In breastfed newborns it may cause **hemorrhagic disease of the newborn**. Intracranial, gastrointestinal and skin bleeding, can occur in vitamin K-deficient infants 1–7 days after birth. Thus, vitamin K (1 mg IM) is given routinely to newborn babies to prevent hemorrhagic disease.

WATER-SOLUBLE VITAMINS—VITAMIN B COMPLEX

Thiamine (Vitamin B₁)

- Thiamine was the first B complex vitamin identified and is referred to as vitamin B₁.

Functions

- Thiamine functions as a coenzyme in many α -ketoacid decarboxylation and transketolation reactions. Inadequate thiamin results in inadequate adenosine triphosphate synthesis and abnormal carbohydrate metabolism, respectively.
- May have an additional role in neuronal conduction.

Deficiency

Causes

- Most dietary deficiency of thiamine is due to **poor dietary intake**. Alcoholism, chronic renal dialysis and chronic illnesses, such as cancer are common precipitant factors. High carbohydrate intake increases need for B₁. Alcohol interferes with the absorption of thiamine and with the synthesis of thiamine pyrophosphate.
- Women with **prolonged hyperemesis gravidarum** can develop thiamine deficiency. Maternal thiamine deficiency can lead to infantile beriberi in breast-fed children.
- Anorexia.
- Patients.
 - With overall poor nutritional status on parenteral glucose.
 - After bariatric bypass surgery.
 - On chronic diuretic therapy due to increased urinary thiamine losses.

Effects of Deficiency

- **Mild deficiency:** Thiamine deficiency in its early stage is characterized by irritability, decrease in short-term memory, anorexia, fatigue and headaches.
- **More severe deficiency—beriberi:** Prolonged thiamine deficiency causes beriberi. It is classically categorized as **wet or dry or combination of two**. It is the classic deficiency syndrome observed in **individuals consuming polished rice diet**. It shows combinations of peripheral neuropathy, cardiovascular dysfunction and cerebral dysfunction.
 - **Peripheral neuropathy:** Complain of pain and paresthesia associated with diminished reflexes. The neuropathy affects the legs most markedly, and these patients have difficulty rising from a squatting position.
 - **Cardiovascular dysfunction (“wet beriberi”):** Congestive heart failure and low peripheral vascular resistance.
 - **Cerebrovascular dysfunction:**
 - ♦ Wernicke's encephalopathy: Acute appearance of nystagmus, ophthalmoplegia, ataxia and psychotic

symptoms. The acute symptoms are reversible when treated with thiamine. However, if untreated, they may be followed by a prolonged and largely irreversible condition, called Korsakoff syndrome.

- ◆ **Korsakoff syndrome:** Characterized clinically by hallucinations, disturbances of short-term memory and confabulation. The syndrome is common in chronic alcoholics but may also be seen with thiamine deficiency resulting from gastric disorders, including carcinoma, chronic gastritis, or persistent vomiting.
- **Wet beriberi** presents primarily with cardiovascular symptoms.
- **Dry beriberi** presents with a symmetric peripheral neuropathy of the motor and sensory systems with diminished reflexes.

Riboflavin (Vitamin B₂)

- It is important for the metabolism of fat, carbohydrate, and protein. It also plays a role in drug and steroid metabolism, including detoxification reactions.
- Serves as a coenzyme for a diverse array of biochemical reactions and as an electron donor.
- The primary coenzymatic forms of riboflavin are flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) and are known as *flavoenzymes* (e.g. succinic acid dehydrogenase, monoamine oxidase, glutathione reductase).

Deficiency

Causes: Almost always is due to dietary deficiency and is usually seen in conjunction with deficiencies of other B vitamins.

Effects of Deficiency

- Nonspecific and mainly manifests as **lesions of the mucocutaneous surfaces of the mouth and skin**. These include **hyperemia and edema of nasopharyngeal mucosa, cheilosis, angular stomatitis, glossitis and seborrheic dermatitis**. Other lesions include corneal vascularization, normochromic-normocytic anemia and personality changes.

Niacin (Vitamin B₃)

Q. Write short essay/note on niacin deficiency.

- The term *niacin* refers to nicotinic acid and the corresponding amide, nicotinamide and their biologically active derivatives.

- Nicotinic acid and nicotinamide serve as precursors of two coenzymes, nicotinamide adenine dinucleotide (NAD) and NAD phosphate (NADP), which are important in numerous oxidation and reduction reactions.
- NAD and NADP are active in adenine diphosphate-ribose transfer reactions involved in DNA repair and calcium mobilization.

Deficiency

Pellagra

- Niacin deficiency causes **pellagra**. It is found mostly in populations in which corn is the major source of energy in parts of China, Africa and India.
- **Early symptoms:** Loss of appetite, generalized weakness and irritability, abdominal pain, and vomiting.
- **Early signs:** Bright red glossitis, stomatitis, vaginitis, esophagitis, vertigo and burning dysesthesias.
- **Advanced stages:** Characteristic skin rash develops that is pigmented and scaling that develops in skin areas exposed to sunlight. This rash is known as **Casal's necklace** because it forms a ring around the neck.
- **Four Ds:** Diarrhea (in part due to proctitis and in part due to malabsorption), depression, seizures and dementia (or associated symptoms of anxiety or insomnia) leading to death and dermatitis, are part of the pellagra syndrome.

Pyridoxine (Vitamin B₆)

- *Vitamin B₆* refers to several derivatives of pyridine that include pyridoxine (PN), pyridoxal (PL) and pyridoxamine (PM), which are interconvertible in the body. The coenzymatic forms are pyridoxal-5-phosphate (PLP) and pyridoxamine-5-phosphate (PMP). 5'-Pyridoxal phosphate (PLP) is a cofactor for more than 100 enzymes involved in amino acid metabolism.
- Vitamin B₆ is also involved in synthesis of heme and many neurotransmitters and in the metabolism of glycogen, lipids, steroids, sphingoid bases and several vitamins, including the synthesis of niacin from tryptophan.

Deficiency

- Deficiency usually seen in conjunction with other water-soluble vitamin deficiencies.
- Certain medications, such as isoniazid, cycloserine, penicillamine, L-dopa, ethanol and theophylline can inhibit B₆ metabolism. Pyridoxine should be given concurrently with isoniazid to avoid neuropathy. Because vitamin B₆ interferes with the action of L-dopa, it should not be given with this drug.

Effects of Deficiency

- **Stomatitis, angular cheilosis, glossitis, irritability, depression and confusion** occur in moderate to severe depletion.
- **Microcytic hypochromic anemia** is due to diminished hemoglobin synthesis, since it is the first enzyme involved in heme biosynthesis. It may also produce normochromic-normocytic anemia.
- **In infants: Diarrhea, seizures/convulsions and anemia.**
- **Severe vitamin B₆ deficiency: Peripheral neuropathy and abnormal electroencephalograms.**

Vitamin B₁₂

A group of closely related cobalamine compounds.

Functions

Vitamin B₁₂ is indirectly **required for DNA synthesis** in various metabolic steps and its deficiency impairs DNA synthesis. The two active coenzyme forms are deoxyadenosylcobalamin and methylcobalamin.

- Methylcobalamin is the main form of vitamin B₁₂ in plasma, and is an essential coenzyme for conversion of homocysteine to methionine and formation of tetrahydrofolate (THF) from methyl THF.
- Vitamin B₁₂ is also required for conversion of methylmalonyl CoA to succinyl malonyl CoA.

Deficiency

Causes (Refer Box 10.3)

- Dietary inadequacy is a rare cause of deficiency except in strict vegetarians.
- Mostly due to loss of intestinal absorption. These include pernicious anemia, pancreatic insufficiency, atrophic gastritis, small bowel bacterial overgrowth, or ileal disease.

Effects of Deficiency

- **Hematological changes: Megaloblastic anemia** (refer pages 256–260) and megaloblastic changes in other epithelia.
- **Neurologic complications: Demyelination of peripheral nerves**, posterior and lateral columns of spinal cord, and nerves within the brain. Altered mentation, depression, and psychoses occur.

Folic Acid

- Folates are a group of related pterin compounds. The fully oxidized form is called folic acid, which is not found in nature but is the pharmacologic form of the vitamin.

Functions

- All folate functions relate to its ability to transfer one-carbon groups.
- The active form of folic acid is tetrahydrofolate [THF] which is the biologic “middleman” involved in metabolic processes which synthesize DNA.

Deficiency

Causes (refer Box 10.3)

Effects of Deficiency

Megaloblastic anemia (refer pages 256–260), diarrhea.

Vitamins and their principal clinical manifestations are summarized in Table 9.1.

PROTEIN–ENERGY MALNUTRITION

- **Protein–energy malnutrition (PEM) or protein–calorie malnutrition** refers to a group of malnutrition where there is inadequate calorie or protein intake.
- Severe PEM is a serious, often lethal disease and usually affects children of low-income countries.
- **PEM include marasmus, kwashiorkor and intermediate states of marasmus–kwashiorkor.**

Marasmus

Q. Write short note on marasmus.

- *Marasmus* is the childhood form of starvation. It develops **due to inadequate intake of protein and calories** and is characterized by emaciation. It is characterized by **emaciation** with obvious muscle wasting and loss of body fat. There is **no edema**. The hair is thin and dry.
- The marasmic child does not appear as apathetic or anorexic as with kwashiorkor. Diarrhea occurs frequently and there may be signs of infection.

Kwashiorkor

- **Inadequate protein intake:** Kwashiorkor develops due to an inadequate protein intake with reasonable caloric (energy) intake.
- **Edema:** In kwashiorkor, marked protein deprivation causes hypoalbuminemia leading to **generalized or dependent edema**. Edema is not a characteristic of marasmus.
- **Skin lesions:** Children with kwashiorkor have characteristic *skin lesions*. This consists of **alternating zones of hyperpigmentation, and hypopigmentation, producing “flaky paint” appearance.**

TABLE 9.1: Vitamins and their principal clinical manifestations

Vitamin	Clinical finding
Thiamine	Beriberi (dry or wet): Neuropathy, muscle weakness and wasting, cardiomegaly, edema, ophthalmoplegia, confabulation
Riboflavin	Magenta tongue (glossitis), angular stomatitis, seborrhea, cheilosis and seborrheic dermatitis
Niacin	Pellagra : Pigmented rash of sun-exposed areas, bright-red tongue, diarrhea, apathy, memory loss, disorientation
Vitamin B ₆	Seborrhea, glossitis, convulsions, neuropathy, depression, confusion, anemia
Folate	Megaloblastic anemia, atrophic glossitis
Vitamin B ₁₂	Megaloblastic anemia, loss of vibratory and position sense, abnormal gait, dementia
Vitamin C	Scurvy: Petechiae, ecchymosis, inflamed and bleeding gums, joint effusion, poor wound healing, fatigue
Vitamin A	Xerophthalmia, night blindness, Bitot's spots, follicular hyperkeratosis, immune dysfunction
Vitamin D	Rickets in children: Skeletal deformation, rachitic rosary, bowed legs. Osteomalacia in adults
Vitamin E	Peripheral neuropathy, spinocerebellar ataxia, skeletal muscle atrophy, retinopathy
Vitamin K	Elevated prothrombin time, bleeding

Q. Write short essay/note on flag sign.

- **Hair changes:** These include **loss of color or alternating bands of pale and darker hair**.
- **Other features:** The other features that differentiate kwashiorkor from marasmus are as follows:
 - Presence of enlarged, fatty liver.
 - Development of apathy, listlessness and loss of appetite.
 - Likely presence of vitamin deficiencies.
 - Defects in immunity and secondary infections.

MORPHOLOGY

1. Growth failure.
2. Peripheral edema in kwashiorkor.
3. Loss of body fat and atrophy of muscle more marked in marasmus.

Differences between kwashiorkor and marasmus are listed in Table 9.2.

TABLE 9.2: Differences between kwashiorkor and marasmus

Feature	Kwashiorkor	Marasmus
Definition	Inadequate protein intake with reasonable caloric (energy) intake	Inadequate intake of both protein and calories
Age	Children 6 months to 3 years	Infants under 1 year
Growth failure	Present	Present
Edema	Localized or generalized	Absent
Liver	Enlarged fatty	Not enlarged

Cachexia

- PEM is a common complication that develops in patients with AIDS or advanced cancers. In these settings it is called as cachexia.
- Cachexia occurs most commonly in patients with cancers of gastrointestinal, pancreatic and lung.
- Characterized by **extreme weight loss, fatigue, muscle atrophy, anemia, anorexia and edema**.

OBESITY**Q Write short essay/note on obesity.**

Definition: Obesity is defined as an accumulation of excess body fat (adipose tissue) that is of sufficient magnitude to impair health.

Prevalence of obesity: Obesity is a major health problem in developed countries and an emerging health problem in developing countries, such as India.

Classification of overweight and obesity by body mass index is presented in Table 9.3.

TABLE 9.3: Classification of overweight and obesity by body mass index

	BMI Kg/m ²
Normal	18.5–24.9
Overweight	25.0–29.9
Obesity—Class I	30.0–34.9
Obesity—Class II	35.0–39.9
Extreme obesity—Class III	≥40

Types of Obesity

The distribution of the stored fat is important in obesity and **according to body fat distribution** obesity is divided into:

- **Central ('abdominal', 'visceral', 'android' or 'apple-shaped') obesity:** This type of obesity shows increased accumulation of fat in the trunk and in the abdominal cavity/ intra-abdominal (in the mesentery and around viscera). It is associated with a greater risk for several diseases (e.g. Type 2 diabetes, the metabolic syndrome and cardiovascular disease) than generalized obesity.
- **Generalized' ('gynoid' or 'pear-shaped') obesity:** This type is characterized by excess accumulation of fat diffusely in the subcutaneous tissue

Etiology

Accumulation of fat in obesity can be considered to be the result of caloric imbalance between the energy consumption (intake of calories) in the diet and energy expenditure through exercise and bodily functions. However, the pathogenesis of obesity is complex and incompletely known.

1. Genetic Aspects of Human Obesity

- Obesity is a **polygenic disorder**, with small contributions from a number of different genes.
- Single-gene (**monogenic forms**) disorders are rare and produce severe childhood obesity. These include mutations in the leptin gene and leptin receptor gene, mutations of *POMC* (Proopiomelanocortin), *Mc4R* (melanocortin-4 receptor) *genes*.
- A few **genetic conditions in which obesity** is a feature include the Prader-Willi and Laurence-Moon-Biedl syndromes.

2. Environmental Contributors to Human Obesity

- **Food:** Many environmental factors can influence food intake. Increased consumption of energy-dense foods, larger food portion size, and increased variety of food, increased availability, reduced cost and increased caloric beverages (soft drinks, juices) promote obesity.
- **Physical activity:** It can be divided into three categories: (i) exercise (fitness and sports-related activities); (ii) work-related physical activity; and (iii) non-exercise, non-employment (spontaneous) activity. Increased sedentary behavior, reduced activities of daily living and decreased employment physical activity promote obesity.

Pathogenesis

Body weight regulation (regulation of energy balance) or dysregulation depends on a complex interplay of both humoral (endocrine) and neural mechanism that control appetite and satiety. These neurohumoral mechanisms regulate energy balance and respond to genetic, nutritional, environmental, and psychologic signals. They trigger a metabolic response through the stimulation of centers in the hypothalamus and ultimately influence the effector arms of energy intake and expenditure.

Neurohumoral mechanisms can be subdivided into three components (Fig. 9.4).

1. Peripheral or Afferent System

Peripheral afferent system can be further subdivided into peripheral appetite suppressing signals and peripheral appetite stimulant signals.

- **Peripheral appetite suppressing signals:**

- **Leptin** (Greek term leptos, meaning 'thin.'): It is a **hormone secreted by fat cells** and it stimulates POMC/CART pathway (Fig. 9.4) and inhibits NPY/AgRP pathway and **appetite is suppressed** (anorexigenic). Increased leptin **stimulates physical activity**, heat **production** (thermogenesis) and **energy expenditure**.
- **Adiponectin:** It is a hormone (**fat-burning molecule**) and the 'guardian angel against obesity,' and is

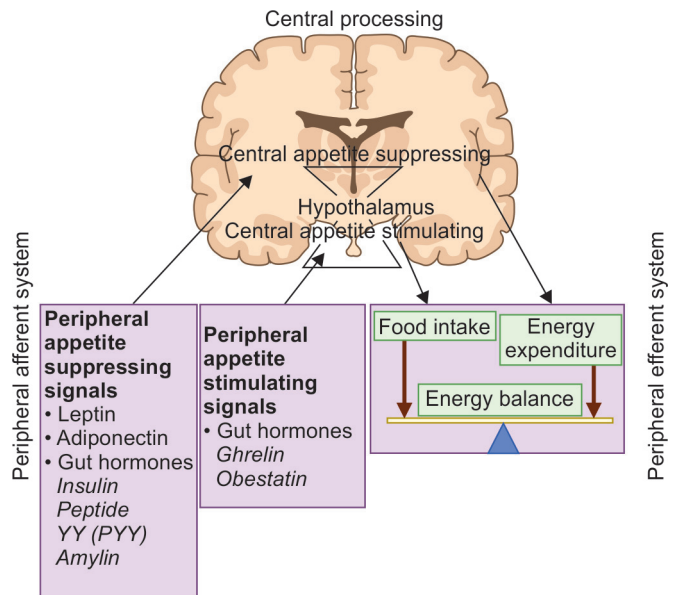


Fig. 9.4: Regulation of energy balance. Peripheral afferent system (appetite suppressing and stimulating signals) influences the activity of the hypothalamus, which is the central regulator of appetite and satiety. Signals from hypothalamus in turn act on peripheral efferent system (food intake and energy expenditure). See text for details

produced mainly by fat cells (adipocytes). Its levels are lower in obese.

- **Resistin:** Primarily produced by macrophages and not fat cells. It **causes insulin resistance**.
- **Gut hormones:** These include ghrelin, PYY, pancreatic polypeptide, insulin and amylin.
 - ♦ **Insulin:** It is **secreted by cells of the pancreas** and act centrally to **activate the appetite suppressing pathway**.
 - ♦ **Peptide YY (PYY):** It is **secreted by the endocrine cells (L cells)** in the ileum and colon. It **reduces appetite**. Other peripheral **appetite suppressing** signals include glucagon-like peptide 1 (GLP1) and oxyntomodulin.
 - ♦ **Amylin:** It is a peptide secreted with insulin from pancreatic β -cells.
- **Peripheral appetite stimulating signals**
 - Gut hormones
 - ♦ **Ghrelin:** It is produced by the **oxyntic cells of the fundus of the stomach** and in the **arcuate nucleus of the hypothalamus**. Ghrelin **increases food intake** (orexigenic effect) and **stimulates appetite** by activating the central appetite stimulating NPY/AgRP pathway.
 - ♦ **Obestatin:** It is a peptide produced by the **same gene that encodes ghrelin**. It counteracts the **increase in food intake induced by ghrelin**.
 - **Retinol binding protein 4 (RBP4):** Secreted by fat cells. Its actions counteract with those of insulin. Raised levels of RBP4 found in Type 2 diabetes mellitus.

2. Central Processing

The arcuate nucleus of the hypothalamus processes integrates neurohumoral peripheral afferent signals and generates efferent signals. It consists of:

- **Central appetite suppressing (anorexigenic pathway or leptin melanocortin pathway):** In this pathway, **POMC/CART neurons enhance energy expenditure** and weight loss through the production of the anorexigenic α MSH (α -melanocyte stimulating hormone) by cleavage of POMC by PC1 (prohormone convertase). It exerts its **appetite suppressing action via the activation of the melanocortin receptors 3 and 4 (MC3/4R)** in second-order neurons. Second order neurons in turn produce factors, such as thyroid releasing hormone (TSH) and corticotropin releasing hormone (CRH) that increase the basal metabolic rate and anabolic metabolism, thus **regulate food intake favoring weight loss**.
- **Central appetite stimulating (orexigenic) pathway:** It consists of: (i) NPY/AgRP neurons promote food intake (orexigenic effect) and weight gain, through the

activation of Y1/5 receptors in secondary neurons. (ii) Secondary neurons in turn release factors, such as melanin-concentrating hormone (MCH) and orexin, which stimulate appetite. This pathway also decreases energy expenditure.

These pathways interact with each other and communicate with other parts of the brain, and influence the autonomic nervous system and ingestive behavior. These central pathways are in turn influenced by a variety of peripheral signals discussed above.

3. Peripheral Efferent System

It is organized into two pathways namely anabolic and catabolic that control food intake and energy expenditure, respectively.

a. Energy intake (food intake):

- **Food:** The increase in obesity can be related to the type of food consumed (i.e. food containing sugar and fat) and also psychological factors.
- **Control of appetite: Signals may affect different aspects of eating behavior.** For example, ghrelin (peptide produced by the stomach) increases hunger but does not affect satiation or satiety. Cholecystokinin causes satiation, **but has no effect on satiety**. Leptin act on multiple pathways, **its deficiency causes increased hunger and reduced satiation**
- Following a meal, substances such as cholecystokinin (CCK), bombesin and glucagon-like peptide 1 (GLP1) are released from the small intestine and glucagon and insulin from the pancreas. These hormones are involved in the control of satiety. The control of appetite is extremely complex. Many transmitters in the central nervous system affect appetite:
 - **Appetite inhibitors:** Dopamine, serotonin, γ -aminobutyric acid
 - **Appetite stimulators:** For example, opioids
 - **Regulation of food intake by central nervous system.**

b. Energy expenditure:

It can be divided into resting (or basal) metabolic rate, the thermic effect of food, and physical activity energy expenditure.

- **Resting basal metabolic rate (BMR):** BMR is the energy expenditure and accounts for about 70% of daily energy expenditure, whereas active physical activity contributes to 5–10% of energy expenditure.
- **Thermic effect of food (thermogenesis):** About 10% of ingested energy is spent in the process of digestion, absorption and metabolism of nutrients irrespective of physical activity. This is called as dietary induced

thermogenesis which is lower in obese and post-obese individuals.

- **Physical activity:** Obese individuals tend to spend more energy during physical activity as they have a larger mass to move.

Pathologic Consequences of Obesity (Complications of Obesity)

- Morbidity and mortality:** Obesity has many adverse effects on health and is associated with an increase in mortality and morbidity. Obese individuals are at risk of early death, mainly from diabetes, coronary heart disease and cerebrovascular disease.
- Metabolic complications of obesity:** Central obesity or upper body fat distribution is associated with increased concentration of FFA which can produce several metabolic complications of obesity.
 - **Insulin resistance and Type 2 diabetes mellitus:** Insulin resistance is the decrease/failure of target (peripheral) tissues to insulin action. The skeletal muscle is the main site of insulin stimulated glucose uptake, oxidation and storage. The liver is the main site of glucose production. Normally, insulin promotes glucose utilization (i.e. glucose uptake, oxidation and storage) as well as to inhibit the release of glucose into the circulation. Insulin resistance can develop in obesity and may produce type 2 diabetes mellitus. **Central/upper body/visceral obesity are found in more than 80% of patients with type 2 diabetes.**
 - **Dyslipidemia:** Upper body obesity and type 2 diabetes mellitus are associated with an atherogenic lipid profile. Dyslipidemia includes increased triglycerides, increased low-density lipoprotein (LDL) cholesterol with very-low-density lipoprotein (VLDL) cholesterol, decreased high-density lipoprotein (HDL) cholesterol, and decreased levels of the vascular protective adipokine adiponectin. Dyslipidemia increases the risk of cardiovascular diseases (atherosclerosis, cardiomyopathy) in the metabolic syndrome.
- Endocrine manifestations of obesity:**
 - **Women:** Polycystic ovarian syndrome (PCOS) and menstrual abnormalities.
 - **Men:** Reduced plasma testosterone and sex hormone-binding globulin (SHBG), increased estrogen levels and gynecomastia.
- Mechanical complications of obesity:**
 - **Osteoarthritis:** Excessive body weight in obesity predisposes to degenerative joint disease (osteoarthritis) and also gout.

- **Venous stasis/varicose veins**
- **Acanthosis nigricans:** It manifests as darkening and thickening of the skinfolds on the neck, elbows and dorsal interphalangeal spaces. It reflects the severity of underlying insulin resistance.
- **Increased friability of skin:** Especially in skinfolds, thereby increasing the risk of fungal and yeast infections.
- **Urinary incontinence.**

e. Pulmonary disease:

- **Obesity hypoventilation syndrome** (Pickwickian syndrome) may also develop.
- **Hypersomnolence:** Develops both at night and during the day. It is often associated with apneic pauses during sleep (sleep apnea), polycythemia and right-sided heart failure (*cor pulmonale*).

f. Cancer:

- Obesity in males is associated with higher mortality from cancer, such as cancer of the prostate, colon, esophagus, rectum, pancreas and liver.
- Obesity in females is associated with higher mortality from cancer of the breasts, endometrium, thyroid, gallbladder, bile ducts, cervix, and ovaries.

g. Gastrointestinal disorders:

- **Gastroesophageal reflux disease**
- **Gallstones:** Higher incidence of gallstones, especially cholesterol gallstones.
- **Fatty liver (steatosis) and nonalcoholic steatohepatitis (NAFLD):** Nonalcoholic steatohepatitis can progress to hepatic cirrhosis and rarely to hepatocellular carcinoma.

EFFECTS OF TOBACCO

Q. Write short essay/note on effects of tobacco.

Smoking is the most prevalent and preventable cause of death. It is the leading exogenous cause of human cancers. Tobacco may be used either for smoking (most commonly as cigarette smoking) or as smokeless tobacco (e.g. snuff, chewing tobacco). The tobacco products as well as exposure to environmental tobacco smoke (passive smoke inhalation termed “second-hand smoke”) can cause lung cancer in nonsmokers. Tobacco effects are dose dependent and cessation of smoking greatly reduces the risk.

Constituents of Tobacco

- Tobacco contains about 2000–4000 substances and more than 60 have been identified as carcinogens (refer Table 16.5).
- **Nicotine** is an alkaloid present in tobacco leaves. It does not directly cause tobacco-related diseases, but is strongly

addictive. Nicotine binds to nicotinic acetylcholine receptors in the brain, and release catecholamines from sympathetic neurons. This is responsible for the acute ill effects of smoking namely increase in heart rate, blood pressure, cardiac contractility and output.

Diseases Caused

Respiratory System

- **Lung cancer:** Components of cigarette smoke, **particularly polycyclic hydrocarbons and nitrosamines** are directly involved in the development of lung cancer in humans (refer Chapter 16). Cytochrome P-450 phase I enzymes (CYPs) and phase II enzymes increase the water solubility of the carcinogens, thereby helping in their excretion. However, few intermediates produced by CYPs are electrophilic and combine with DNA to form DNA adducts. If DNA adducts persist, they can cause mutations in oncogenes and tumor suppressors. The risk of developing lung cancer depends on the number of pack years or cigarettes smoked per day and duration of smoking habit. Smoking also increases the risk of other carcinogens (e.g. asbestos, uranium).
- **Chronic bronchitis, emphysema and chronic obstructive pulmonary disease:** Contents in tobacco smoke directly irritate the tracheobronchial mucosa, producing inflam-

mation and increased mucus production (bronchitis). Cigarette smoke also recruits leukocytes to the lung, and increases the local production of elastase. This injures lung tissue leading to emphysema.

Other Systems

Apart from lung cancer, smoking is a risk factor for many other malignant and nonmalignant disorders of many organ systems.

- **Oral cancers:** Smokeless tobacco along with alcohol consumption is important cause of oral cancer (refer Chapter 17).
- **Other cancers:** Cigarette smoking is associated with **cancers of the esophagus, larynx, pancreas, bladder, kidney, cervix and bone marrow**. Tobacco consumption interacts with alcohol in multiplying the risk of oral, laryngeal and esophageal cancer.
- **Atherosclerosis** (refer Chapter 14): Cigarette smoking is major risk factor of *atherosclerosis* and its major complication, myocardial infarction. Smoking is a risk factor for peripheral vascular disease and cerebrovascular disease.
- **Peptic ulcer disease** (refer Chapter 18).
- **Maternal smoking:** Increases the risk of spontaneous abortions and pre-term births and results in intrauterine growth retardation.

2

SECTION

Hematology and Clinical Pathology

- 10. Disorders of Red Cells
- 11. Disorders of White Cells
- 12. Disorders of Hemostasis
- 13. Clinical Pathology

Disorders of Red Cells

ANEMIA

Q. Define anemia.

DEFINITION

- Anemia is defined as the **decrease** below normal limit (below the reference level for the age and sex of the individual) of the **hemoglobin concentration, erythrocyte count or hematocrit** (ratio of packed red cells to total blood volume).
- It can also be defined as a **reduction of the total circulating red cell mass** below normal limits.
- Functionally**, it is defined as the **decrease in the oxygen-carrying capacity** of the blood, which leads to tissue hypoxia.

Anemia may be **absolute** (decreased RBC mass), or **relative** (associated with a higher plasma volume). Anemia is conventionally used for absolute anemia.

Anemia is characterized by decreased oxygen carrying capacity of blood. Shows decreased Hb and PCV.

WHO criteria for anemia: Adult males Hb <13 g/dL and adult female Hb <12 g/dL.

Classification of Anemia

- Morphological classification** (Table 10.1): It is based on:
 - Red cell size** (normocytic, microcytic, or macrocytic), and
 - Degree of hemoglobinization** (normochromic or hypochromic).
- Etiological classification:** The etiological classification of anemia is presented in Box 10.1.

Classification: Anemias are mainly classified based on (1) morphology and (2) etiology.

Grading of anemia: Mild (Hb 9.1–10.5 g/dL), moderate (Hb 6.0–9.0 g/dL) and severe (Hb < 6.0 g/dL).

Red Cell Indices

Q. Write short notes on red cell indices.

Red cell indices: MCV, MCH, MCHC and RDW.

Red cell indices are useful in morphological characterization and diagnosis of anemias. They are either directly measured or automatically calculated by specialized instruments. Red cell indices include:

Q. Write short notes on mean corpuscular volume.

1. Mean corpuscular volume

- Mean corpuscular volume (MCV) is indicative of average volume of the RBC and is expressed in femtoliters (fL).
- It is used for classification and differential diagnosis of anemias.
- Normal range: 82–98 fL.**




$$\text{MCV} = \frac{\text{PCV} \times 1000}{\text{RBC count in millions}} = \frac{0.45 \times 1000}{5} = 90 \text{ fL}$$

Microcytic anemia have MCV <80 fL and macrocytic anemia have MCV >100 fL.

2. Mean corpuscular hemoglobin

- Mean corpuscular hemoglobin (MCH) indicates the amount of Hb (weight) per RBC and is expressed as picograms (1 pg = 10⁻¹² g).

TABLE 10.1: Morphological classification of anemia**Q. Classify anemia.**

Type of anemia	Microcytic hypochromic	Normocytic normochromic	Macrocytic
Size of RBCs	Smaller than normal	Normal	Larger than normal
Central pallor in RBCs	More than 1/3	Normal	Normal
Mean corpuscular volume (MCV)	Reduced (<80 fL)	Normal (82–98 fL)	Increased (>100 fL)
Mean corpuscular hemoglobin concentration (MCHC)	Reduced (<30 g/dL)	Normal (31–36 g/dL)	Normal (31–36 g/dL)
Examples	Iron deficiency anemia, thalassemia	During blood loss, anemia of chronic diseases	Deficiency of vitamin B ₁₂ and folic acid
Morphology of RBC			

Spurious anemia is the term used when RBC concentration decreases due to hemodilution as seen in third semester of pregnancy.

- It is of limited value in differential diagnosis of anemias.
- **Normal range: 27–32 pg**

$MCH = Hb \text{ (in g/L)} / RBC \text{ (in millions/}\mu\text{L)} = 15 \times 10 / 5 = 30 \text{ pg}$

MCH <26 pg is seen in microcytic anemia and MCH >33 pg is seen in macrocytic anemia.

Q. Write short notes on mean corpuscular hemoglobin.**3. Mean corpuscular hemoglobin concentration**

- Mean corpuscular hemoglobin concentration (MCHC) denotes the average concentration of hemoglobin in the RBC taking volume into account. It is expressed as g/dL (earlier it was expressed as %).
- It is a better indicator of hypochromasia than MCH.
- Normal range: 31–35 g/dL.

$MCHC = Hb \text{ (in g/dL)} / PCV = 15 / 0.45 = 33 \text{ g/dL}$

MCHC <31 g/dL is seen in hypochromic RBC such as iron deficiency anemia (IDA) and thalassemia. MCHC >36 g/dL is an indication of hyperchromic RBCs.

4. Red cell distribution width

- Red cell distribution (RDW) is a quantitative measure of anisocytosis.
- Normal RDW is 11.5–14.5%.
- A normal RDW indicates that RBCs are relatively uniform in size. A raised RDW indicates that red cells are heterogeneous in size and/or shape. In early iron deficiency anemia, RDW increases along with low MCV while in thalassemia trait, RDW is normal with low MCV.

$RDW = (\text{Standard deviation} \div \text{mean cell volume}) \times 100$

RDW is useful for differentiating anemia due to iron deficiency and thalassemia.

ANEMIAS OF IMPAIRED RED CELL PRODUCTION

IRON DEFICIENCY ANEMIA

Q. Discuss the etiopathogenesis of iron deficiency anemia.

Iron deficiency anemia (IDA) is the **most common nutritional disorder**.

Etiology (Box 10.1)

Iron deficiency anemia (IDA) is due to deficiency of iron causing defective heme synthesis.

Pathogenesis of Iron Deficiency Anemia

It is due to decreased synthesis of heme and can be divided into 3 stages.

- **Stage 1 (Iron depletion):** Iron adequate to maintain normal hemoglobin level and **only serum ferritin decreased**.
- **Stage 2 (Iron deficient erythropoiesis):** Lowering of serum iron and transferrin saturation levels without

BOX 10.1: Etiological classification of anemia (according to underlying mechanism)

Q Etiological classification of anemia.

1. Blood Loss

- **Acute:** Trauma
- **Chronic:** Lesions of gastrointestinal tract (e.g. carcinoma colon), gynecological disorders

2. Impaired Red Cell Production

- **Nutritional deficiencies**
 - *Deficiencies affecting hemoglobin synthesis:* Iron deficiency
 - *Deficiencies affecting DNA synthesis:* Megaloblastic anemias due to deficiency or impaired utilization of vitamin B₁₂ and folic acid
 - *Vitamin C deficiency*
- **Inherited genetic defects**
 - Defects affecting erythroblast maturation: Thalassemia syndromes
 - Defects leading to stem cell depletion: Fanconi anemia, telomerase defect
- **Erythropoietin deficiency:** Renal failure, anemia of chronic disease
- **Immune-mediated injury of progenitors:** Aplastic anemia, pure red cell aplasia
- **Inflammation-mediated iron sequestration:** Anemia of chronic disease
- **Primary hematopoietic neoplasms:** Acute leukemia, myelodysplastic syndromes, myeloproliferative disorders
- **Space-occupying marrow lesions:** Metastatic tumors, granulomatous disease
- **Infections of red cell progenitors:** Parvovirus B19 infection
- **Unknown mechanisms:** Endocrine disorders, liver disease

3. Increased Red Cell Destruction (Hemolytic Anemias)

- **Inherited genetic defects**
 - *Red cell membrane disorders:* Hereditary spherocytosis, hereditary elliptocytosis
 - *Enzyme deficiencies*
 - ◆ **Hexose monophosphate shunt enzyme deficiencies:** G6PD deficiency
 - ◆ **Glycolytic enzyme deficiencies:** Pyruvate kinase deficiency, hexokinase deficiency
- **Hemoglobin abnormalities**
 - *Deficient globin synthesis:* Thalassemia syndromes
 - *Structurally abnormal globins (hemoglobinopathies):* Sickle cell disease
- **Acquired genetic defects**
 - *Deficiency of phosphatidylinositol-linked glycoproteins:* Paroxysmal nocturnal hemoglobinuria
- **Antibody-mediated destruction**
 - Hemolytic disease (Rh disease) of the newborn, transfusion reactions, drug-associated, autoimmune disorders (e.g. systemic lupus erythematosus)
- **Mechanical trauma**
 - *Microangiopathic hemolytic anemias:* Hemolytic uremic syndrome, disseminated intravascular coagulation, thrombotic thrombocytopenia purpura
 - *Cardiac traumatic hemolysis:* Defective cardiac valves
- **Infections of red cells:** Malaria, babesiosis
- **Toxic or chemical injury:** Clostridial sepsis, snake venom, lead poisoning
- **Sequestration:** Hypersplenism

Abbreviations: G6PD, glucose-6-phosphate dehydrogenase; PK, pyruvate kinase

Causes of anemia (Box 10.2):

1. Decreased RBC production
2. Increased RBC destruction (hemolysis) or
3. Blood loss.

Anemia is the expression of underlying disease and from treatment point, the cause of anemia must be identified.

Iron deficiency anemia is the most common anemia.

anemia (Hb, MCV and MCH within normal range). Bone marrow shows iron deficient erythropoiesis.

- **Stage 3 (Iron deficiency anemia):** Low serum iron, serum ferritin and transferrin saturation. Impaired hemoglobin production. Morphologically, **first reduction in the size**

(microcytic) and later increase in the central pallor (hypochromia) of RBCs.

Stages of IDA in sequence: absent of iron stores→decreased serum ferritin→decreased serum iron→increased TIBC → decreased iron saturation→ microcytic hypochromic anemia.

BOX 10.2: Causes of iron deficiency anemia

1. **Dietary deficiency/lack**
 - Milk-fed infants
 - Elderly with improper diet and poor dentition
 - Low socioeconomical sections
 - Vegetarians (contains poorly absorbable inorganic iron)
2. **Impaired absorption**
 - Total/partial gastrectomy
 - Intestinal absorption is impaired in sprue, other causes of intestinal steatorrhea and chronic diarrhea
 - Specific items in the diet, like phytates of cereals, tannates, carbonates, oxalates, phosphates and drugs can impair iron absorption
3. **Increased demand/requirement**
 - Growing infants, children and adolescents
 - Pregnancy and lactation
4. **Chronic blood loss: due to bleeding from the**
 - Gastrointestinal tract (e.g. peptic ulcers, gastric carcinoma, colonic carcinoma, hemorrhoids, hookworm infestation or nonsteroidal anti-inflammatory drugs)
 - Urinary tract (e.g. renal or bladder tumors)
 - Genital tract (e.g. menorrhagia, uterine cancer)
 - Respiratory tract (e.g. hemoptysis)

Dietary deficiency is the commonest cause of IDA.

In adult men and postmenopausal women, deficiency may be due to chronic gastrointestinal blood loss.

Iron is absorbed in the duodenum.

Infants who consume large amounts of cow's milk are susceptible to develop IDA.

Laboratory Findings

Q. Discuss the laboratory findings in iron deficiency anemia.

Q. Describe the peripheral blood picture and bone marrow finding in iron deficiency anemia.

Peripheral Blood

- **Hemoglobin and hematocrit (PCV): decreased**
- **Red cell indices:**
 - **MCV:** <80 fL (normal 82–98 fL)
 - **MCH:** <25 pg (normal 27–32 pg)
 - **MCHC:** <27 g/dL (31–36 g/dL)
 - **RDW:** Increased and >15%. It is **earliest sign of iron deficiency** (normal 11.5–14.5%).

MCV, MCH and MCHC are reduced. RDW is raised.

Q. Write short notes on peripheral smear findings in iron deficiency anemia.

- **Peripheral smear** (Figs 10.1 and 10.2):
 - **RBCs:** **Microcytic** (small) and **hypochromic** (pale). Severe anemia shows ring/pessary cells. **Moderate anisocytosis** and **poikilocytosis** pencil/cigar-shaped cells.
 - **WBCs:** Normal; eosinophilia in hookworm infestation.
 - **Platelets:** Normal

- **Reticulocyte count:** Low for the degree of anemia.

Peripheral smear shows microcytic hypochromic RBCs.

BONE MARROW

Bone marrow shows micronormoblastic erythroid hyperplasia. Marrow iron is absent. Prussian blue reaction negative.

- **Cellularity:** Moderately hypercellular.
- **M:E ratio:** varies from 2:1 to 1:2 (normal 2:1 to 4:1).
- **Erythropoiesis:** Hyperplasia and **micronormoblastic maturation**.
- **Myelopoiesis:** Normal.
- **Megakaryopoiesis:** Normal.
- **Absence of bone marrow iron:** “Gold standard” test, demonstrated by **negative Prussian blue reaction**.

Serum Iron Profile (Table 10.2)

Reduced: Serum iron, ferritin, % transferrin saturation.
Increased: TIBC, TFR and red cell protoporphyrin.

Reticulocyte Hemoglobin

It is **decreased** and is an **early feature of IDA**.

The earliest laboratory indicator of IDA is reduced reticulocyte hemoglobin.

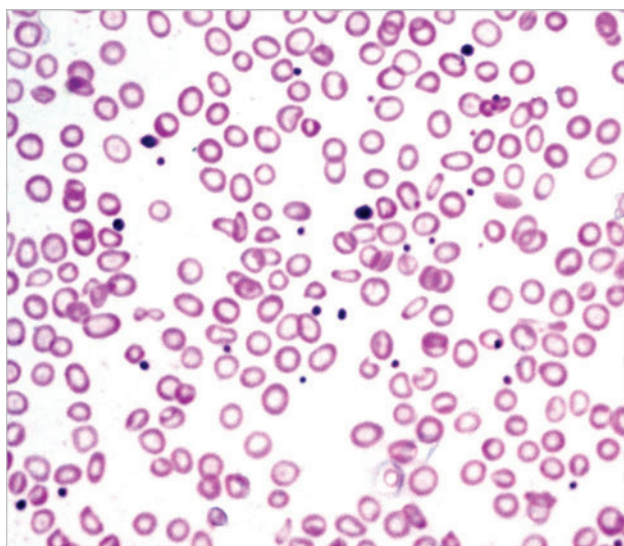


Fig. 10.1: Peripheral blood smear showing microcytic hypochromic red blood cells

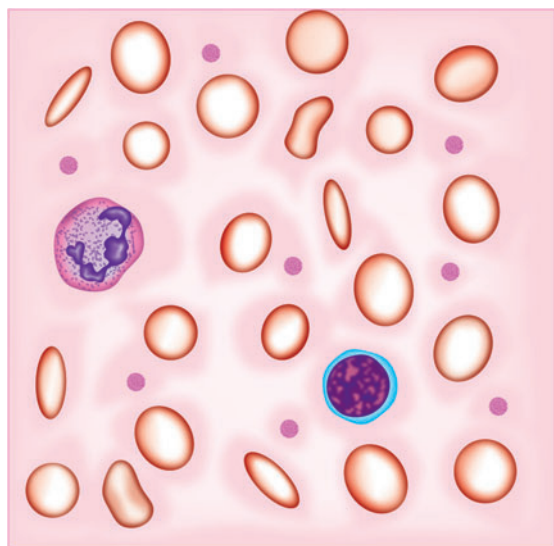


Fig. 10.2: Diagrammatic appearance of peripheral blood smear with microcytic hypochromic red blood cells

Clinical Features of IDA

Q. Mention the various clinical features of iron deficiency anemia.

Nonspecific and related to both severity and the cause of the anemia (e.g. gastrointestinal disease)

- **Onset:** Insidious.
- **Nonspecific symptoms:** Fatigue, palpitations, breathlessness, weakness and irritability.
- **Pharyngeal/esophageal webs** formed cause dysphagia.
- **Patterson-Kelly or Plummer-Vinson syndrome:**
 - Microcytic hypochromic anemia
 - Atrophic glossitis
 - Esophageal webs
- **Congestive heart failure** in severe anemia.
- **Central nervous system:** Pica-unusual craving for substances with no nutritional value like clay or chalk. **Craving for ice (pagophagia) specific to iron deficiency.** Pica may be the cause rather than effect of IDA.

Patterson-Kelly or Plummer-Vinson syndrome: Microcytic hypochromic anemia, atrophic glossitis and esophageal webs.

Physical Findings

Diminished tissue enzymes cause characteristic epithelial changes of iron deficiency anemia.

- Angular stomatitis and glossitis
- Chronic atrophic gastritis
- Koilonychia (spoon nails).

Koilonychia (spoon nails) is a physical finding seen in iron deficiency. First fingernails become thin and flat-platonychia, then brittle and finally spoon-shaped.

Causes of Microcytic Hypochromic Anemia

Q. Enumerate the causes of microcytic hypochromic anemia.

- Iron deficiency anemia
- Thalassemia major and minor

TABLE 10.2: Serum iron profile in Iron deficiency anemia

	Normal range	Value in IDA	Observation
Serum ferritin	15–300 µg/L	<15 µg/L	
Serum iron	50–150 µg/dL	10–15 µg/dL	
Serum transferrin saturation	30–40%	<15%	
Total plasma iron-binding capacity (TIBC)	310–340 µg/dL	350–450 µg/dL	
Serum transferrin receptor (TFR)	0.57–2.8 µg/L	3.5–7.1 µg/L	
Red cell protoporphyrin	30–50 µg/dL	>200 µg/dL	

- **Anemia of chronic disorders**
- **Others:** Alcohol, lead poisoning and drugs
- Sideroblastic anemia (rare cause).

MEGALOBLASTIC ANEMIA

Megaloblastic anemias are characterized by **defective/ impaired DNA synthesis** and **distinct megaloblasts in the bone marrow**. Megaloblastic anemias are common among anemias due to impaired red cell production.

Deficiency of vitamin B₁₂ and folic acid are the main causes of megaloblastic anemia.

Vitamin B₁₂ is present in animal products.

Folic acid is absorbed in the jejunum.

Q. Discuss the causes and pathogenesis of megaloblastic anemia.

Etiology of Megaloblastic Anemia (Box 10.3)

Pathogenesis of Megaloblastic Change

1. **Impaired DNA synthesis:** Megaloblastic anemia is commonly due to deficiency of **vitamin B₁₂ (cyanocobalamin)** or **folic acid**. Both are required for the synthesis of DNA.
 - **Delayed maturation of nucleus.** The **nuclear maturation lags behind the cytoplasmic maturation** and results in **abnormally large nucleated erythroid precursors** named as **megaloblasts**.
 - Cytoplasm matures normally. RBCs are larger than normal → **macrocytes**.
 - Affects all rapidly dividing cells of the body (including skin, gastrointestinal tract, and bone marrow).

Deficiency of vitamin B₁₂ and folic acid → delayed nuclear maturation → megaloblast → macrocyte.

2. **Ineffective erythropoiesis:** Megaloblast precursors undergo intramedullary destruction.

Ineffective erythropoiesis and hemolysis are responsible for anemia.

Laboratory Findings of Megaloblastic Anemia

Q. Write short note on the laboratory findings in megaloblastic anemia.

Blood findings in vitamin B₁₂ and/or folic acid deficiency are similar.

BOX 10.3: Causes of megaloblastic anemia

Vitamin B₁₂ Deficiency

1. **Decreased intake:** Inadequate diet, “pure vegetarians” (vegans)
2. **Impaired absorption**
 - Gastric: Deficiency of gastric acid or pepsin or intrinsic factor
 - Pernicious anemia
 - Post-gastrectomy
 - Intestinal
 - Loss of absorptive surface
 - ♦ Malabsorption syndromes
 - ♦ Diffuse intestinal disease, e.g. lymphoma, systemic sclerosis
 - ♦ Ileal resection, Crohn disease
 - Bacterial or parasitic competition for vitamin B₁₂
 - ♦ Bacterial overgrowth in blind loops and diverticula of bowel
 - ♦ Fish tapeworm infestation (*Diphyllobothrium latum*)

3. **Increased demand:** Pregnancy, hyperthyroidism, disseminated cancer

Folic Acid Deficiency

1. **Decreased intake:** Inadequate diet—alcoholism, malnutrition
2. **Impaired absorption**
 - Malabsorption states: Nontropical and tropical sprue
 - Diffuse infiltrative diseases of the small intestine (e.g. lymphoma)
 - Drugs: anticonvulsant phenytoin and oral contraceptives
3. **Increased loss:** Hemodialysis
4. **Increased demand:** Pregnancy, infancy, disseminated cancer, markedly increased hematopoiesis
5. **Impaired utilization:** Folic acid antagonists, such as methotrexate

Peripheral Blood

- **Hemoglobin and hematocrit (PCV): Reduced**
- **Red cell indices**
 - **MCV:** Above 100 fL (normal 82–98 fL)
 - **MCH** (normal 27–32 pg)
 - **Normal MCHC** (31–36 g/dL)

Q. Write short note on macropolycyte.

Q. Write short note on oval macrocyte.

- **Peripheral smear** (Figs 10.3 and 10.4): **Pancytopenia** (decreased RBC, WBCs and platelets).
 - **RBCs:**
 - ♦ Macrocytic and oval (**egg-shaped macro-ovalocytes**)-**diagnostic**.
 - ♦ Most macrocytes **lack the central pallor** (Figs 10.3 and 10.4).
 - ♦ **Marked** variation in the size and shape of red cells (**anisopoikilocytosis**).

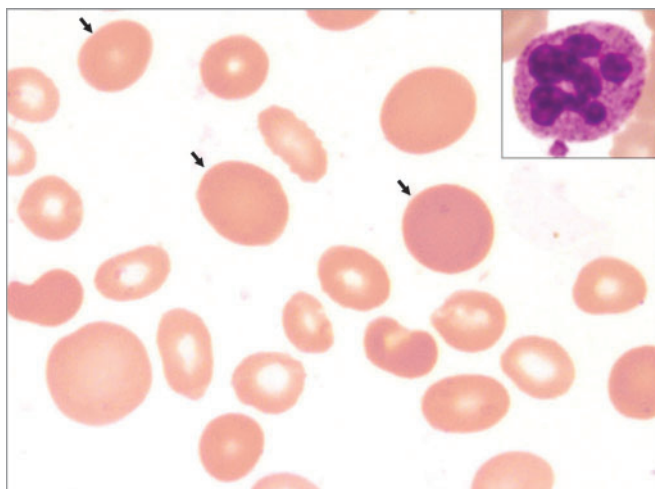


Fig. 10.3: Peripheral blood smear showing macro-ovalocytes (arrows) and hypersegmented neutrophil (inset)

- ◆ Evidence of dyserythropoiesis: Basophilic stippling, Cabot ring and Howell Jolly bodies.
- **WBCs:**
 - ◆ **Decreased WBC count** (leukopenia).
 - ◆ **Hypersegmented neutrophils** (more than five nuclear lobes): First and specific morphological sign of megaloblastic anemia. These neutrophils are also larger than normal (**macropolys**).
- **Platelets: Decreased.**

• **Reticulocyte count:** Normal or low.

Megaloblastic anemia

- Pancytopenia
- Macro-ovalocytes
- Hypersegmented neutrophils
- Macropolys.

In megaloblastic anemia due to vitamin B₁₂ deficiency, reticulocyte count may be normal or low and high reticulocyte count is seen on 7th day following vitamin B₁₂ therapy.

Dimorphic Anemia

- **Combined vitamin B₁₂/folic acid and iron deficiency.**
- Peripheral smear shows two populations of RBCs namely: macro-ovalocytes and microcytic hypochromic (Fig. 10.5).

A mixture of microcytic hypochromic and macrocytic RBCs is termed as dimorphic picture and occurs in mixed deficiency of iron and folic acid or vitamin B₁₂.

BONE MARROW

- **Cellularity:** Moderately to **markedly hypercellular.**
- **M: E ratio:** Due to marked erythroid hyperplasia, M: E ratio is reversed ranging from 1:1 to 1:6 (normal 2:1 to 4:1).

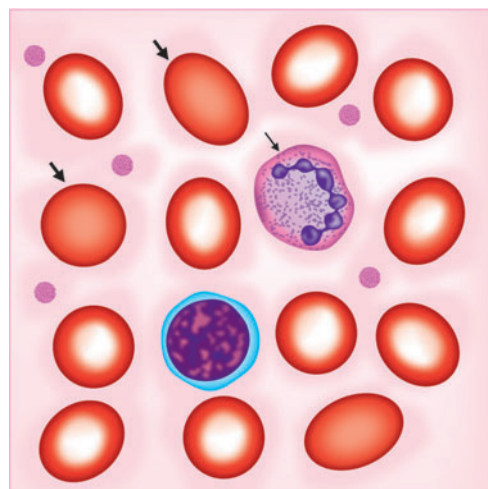


Fig. 10.4: Diagrammatic peripheral blood smear showing macro-ovalocytes (thick arrows) and hypersegmented neutrophil (thin arrow)

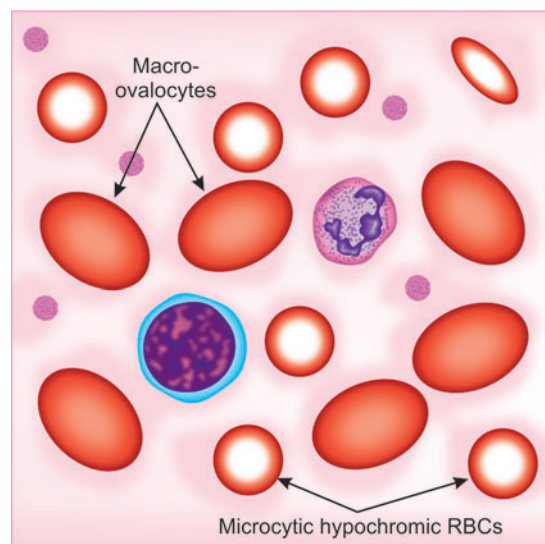


Fig. 10.5: Diagrammatic peripheral blood smear of dimorphic anemia showing macro-ovalocytes and microcytes

Q. Write short note on megaloblast.

- **Erythropoiesis: Megaloblastic type** (Figs 10.6 and 10.7)
 - **Megaloblasts:** Large, abnormal counterparts of normal normoblasts. Megaloblast shows **asynchrony of nuclear and cytoplasmic maturation**. The cytoplasm shows normal hemoglobinization.
 - **Ineffective erythropoiesis:** Developing megaloblasts die in marrow (intramedullary hemolysis).
- **Myelopoiesis:**
 - Myeloid cells adequate in number.
 - Granulocytic precursors display nuclear-cytoplasmic asynchrony in the form of **giant metamyelocytes and band forms**.
- **Megakaryopoiesis:** Normal or increased in number.
- **Bone marrow iron:** Moderately increased.

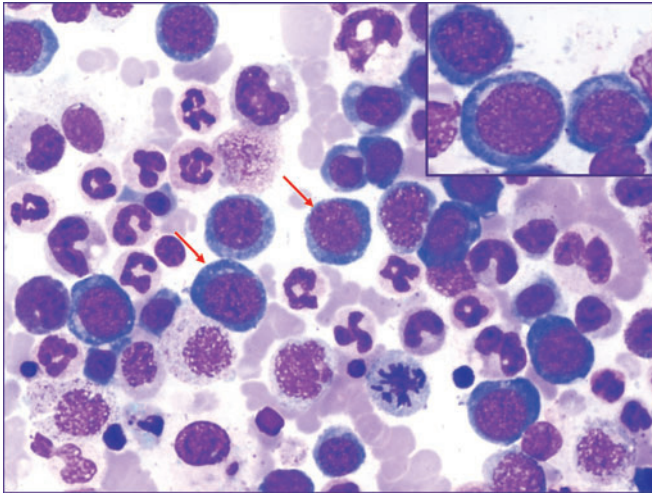


Fig. 10.6: Bone marrow aspirate showing megaloblastic precursors (arrows) in varying stages of maturation (inset shows early megaloblast)

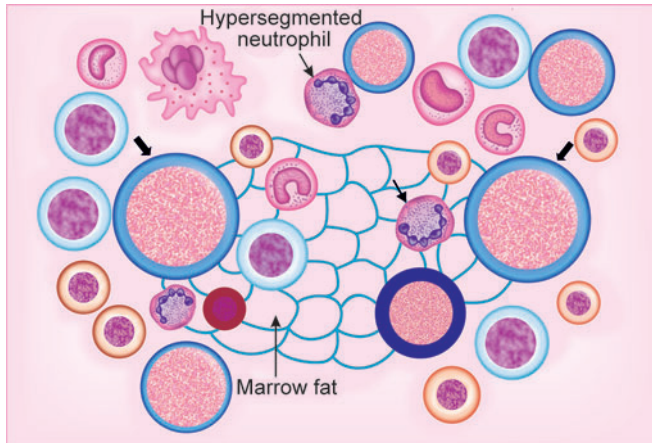


Fig. 10.7: Diagrammatic picture of bone marrow aspirate showing megaloblastic precursors (thick arrows) in varying stages of maturation

Megaloblast are large, abnormal precursors of RBCs seen in the bone marrow of patients with megaloblastic anemia.

The differences between normoblasts and megaloblasts are shown in Table 10.3.

Biochemical Tests for Megaloblastic Anemia

Common for both Vitamin B₁₂ and Folic Acid Deficiency

- Serum homocysteine
- Serum bilirubin: Mild increase causes mild jaundice
- Serum iron and ferritin
- Plasma lactate dehydrogenase (LDH)
- Serum vitamin B₁₂/folate decreased.

Deoxyuridine suppression test is abnormal even before the morphological changes.

Diagnostic Tests for Vitamin B₁₂ Deficiency

- Serum vitamin B₁₂ levels: decreased
 - Serum methylmalonic acid
 - Urinary excretion of methylmalonic acid
- Schilling test for vitamin B₁₂ absorption (Refer page 252).

Deoxyuridine suppression test: It is a sensitive measure of deficiency of 5, 10-methylene THF, which occurs in both folic acid and vitamin B₁₂ deficiency.

Schilling test determines the cause of vitamin B₁₂ deficiency.

Specific Tests for Folic Acid Deficiency

- Serum folic acid levels: decreased
- FIGLU in urine: excessively excreted.

TABLE 10.3: Differences between normoblast and megaloblast.

Q. List the differences between normoblast and megaloblast.

Characteristics	Normoblast	Megaloblast
Cell size	Normal	Larger than corresponding normoblast
Nuclear chromatin	Normal	Open sieve-like
Nuclear maturation	Normal	Lags behind cytoplasmic maturation
Mitosis	Normal	Increased and abnormal
Maturation in bone marrow	Normal (Late > intermediate > early normoblast)	Increased proportion of more primitive erythroid cells (Late < intermediate < early megaloblast)
Evidence of dyserythropoiesis	Absent	Present (irregular nuclei, Howell Jolly bodies)
Myelopoiesis	Normal	Shows giant metamyelocytes
Found in	Normal bone marrow	Bone marrow of megaloblastic anemia

Megaloblasts:

- Nuclear maturation lags behind cytoplasmic maturation.
- Nuclei have open sieve-like chromatin.

Megaloblastic anemia- bone marrow:

- Megaloblasts
- Giant metamyelocytes.

PERNICIOUS ANEMIA

Q. Discuss the etiopathogenesis and morphology of pernicious anemia.

Pernicious anemia (PA) is an autoimmune disease due to **deficiency of intrinsic factor** causing **impaired absorption of vitamin B₁₂** and megaloblastic anemia.

Rare in India. A genetic predisposition is suspected.

Age: older age—**fifth to eighth decades** of life.

Sex: females are more involved than males (F: M is 1.5: 1).

Vitamin B₁₂ is absorbed in terminal ileum and requires IF.

Etiopathogenesis

- An **autoimmune** disease due to destruction of gastric mucosa.
- Stomach shows **damage to parietal cells**, dense infiltration by lymphocytes and plasma cells → **chronic atrophic gastritis** → **failure of production of intrinsic factor**.
- **Presence of autoantibodies: Two major types** of autoantibodies:
 - **Anti-intrinsic factor (IF) antibody**
 - ◆ **Type I (blocking): antibody:** Blocks the binding of vitamin B₁₂ to IF. Present in 50–75% of the cases.
 - ◆ **Type II (binding) antibody:** attaches to the IF–vitamin B₁₂ complex and prevent its binding to receptors in the ileum. Present in about 40% of patients.
 - **Parietal cell (Type III) antibody:** Neither specific for PA nor other autoimmune disorders. It is found in 90% of patients.

Morphology

Alimentary System

- **Atrophic glossitis:** Tongue shiny, glazed and beefy.
- **Stomach:**
 - **Diffuse chronic atrophic gastritis** and impaired secretion of hydrochloric acid, pepsin and intrinsic factor.
 - ◆ Histologically, atrophy of the glands, with loss of both chief cells and parietal cells.
 - ◆ Nuclei of mucosal cells look similar to that of megaloblasts.
 - ◆ Dense infiltration by lymphocytes and plasma cells.
 - **Intestinal metaplasia.**

Central Nervous System

Found in 75% of cases.

- **Demyelination in the dorsal and lateral tracts:** Subacute combined degeneration.
- Peripheral neuropathy.

Laboratory Findings (Fig. 10.8)

Q. Write short note on laboratory findings in pernicious anemia.

Blood, bone marrow and biochemical test findings are similar to those described earlier for megaloblastic anemias (Refer page 256–258).

Specific Diagnostic Tests for Pernicious Anemia

Q. What is Schilling test?

- **Schilling test for vitamin B₁₂ absorption: abnormal?**
 - Radioactive vitamin B₁₂ is used to assess the status of intrinsic factor (IF) and vitamin B₁₂.
 - Helps in distinguishing megaloblastic anemia due to IF deficiency (pernicious anemia) from other causes of vitamin B₁₂ deficiency.
- **Serum antibodies to intrinsic factor** are highly specific for pernicious anemia
- **Achlorhydria** with histamine/pentagastrin stimulation.
- Severe **deficiency of intrinsic factor**.

Clinical Features of Megaloblastic Anemia

Q. Mention the various clinical features of megaloblastic anemia.

The clinical features of vitamin B₁₂ deficiency anemia and pernicious anemia are:

- **Onset: insidious** and progresses slowly.
- **Classic triad of presentation:** weakness, sore throat and paresthesias.
- **Tongue:** Painful red “beefy” tongue.
- **Neurological manifestations:**

Q. Write short note on effects of vitamin B₁₂ deficiency on the nervous system.

- **Bilateral peripheral neuropathy:** Glove and sock distribution of **numbness or paresthesia**
- **Demyelination of spinal cord:** Subacute combined demyelination/degeneration of dorsal and lateral tracts—**ataxia**, uncoordinated gait, impairment of vibration and position sense.

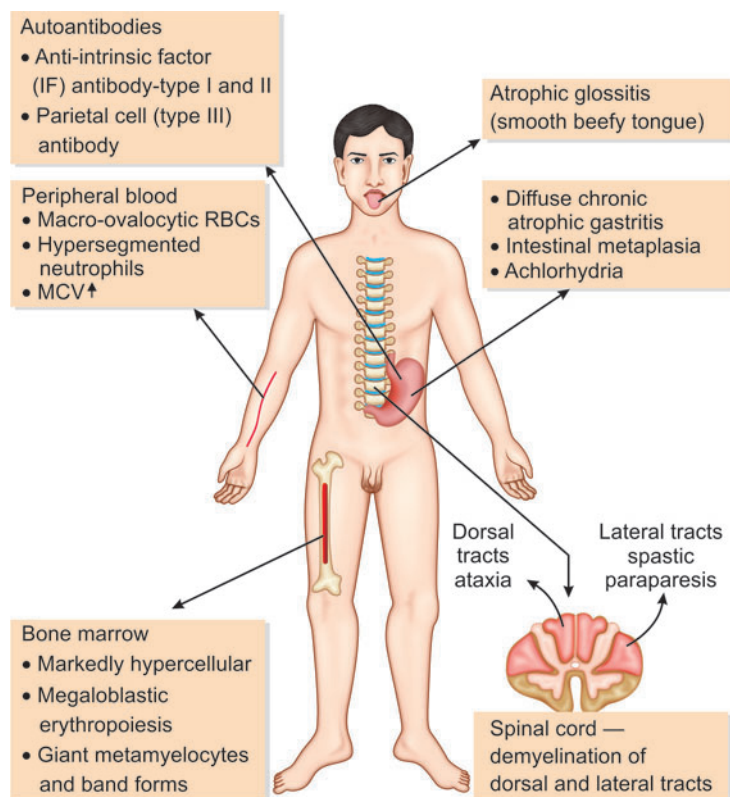


Fig. 10.8: Clinical features and laboratory findings in pernicious anemia

Pernicious anemia (PA) present with features of megaloblastic anemia due to vitamin B₁₂ deficiency. In addition, it may show features of atrophic gastritis and achlorhydria.

PA patients sometimes have a lemon-yellow color owing to a combination of pallor and mild jaundice caused by excess breakdown of hemoglobin.

Atrophic gastritis may predispose to carcinoma stomach.

PA: Autoimmune disease

- Atrophic gastritis
- IF deficiency
- Autoantibodies.

Schilling test: Diagnostic of PA but now very infrequently performed.

- **Atherosclerosis:** Serum homocysteine level is raised and is a risk factor for atherosclerosis and thrombosis.

Folate deficiency anemia presents with features of megaloblastic anemia due to vitamin B₁₂. Unlike with vitamin B₁₂ deficiency, neurological symptoms does not occur.

Q. Write short notes on causes of macrocytic anemia.

Nonmegaloblastic causes of macrocytic anemia:

Megaloblastic anemia: Vitamin B₁₂ and folic acid

1. Alcohol
2. Liver disease
3. Myxedema
4. Cytotoxic drugs
5. Myeloma
6. Aplastic anemia
7. Reticulocytosis
8. Red cell aplasia.

APLASTIC ANEMIA

Q. Write short note on aplastic anemia.

Chronic primary hematopoietic stem cell (HSC) disorder characterized by:

- **Pancytopenia** (anemia, neutropenia and thrombocytopenia).
- With **markedly hypocellular bone marrow** (less than 30% cellularity).

Etiology

Q. Write short note on causes of aplastic anemia.

The most common causes associated with aplastic anemia are shown in Box 10.4.

Pathogenesis (Fig. 10.9)

- Direct damage to the hematopoietic stem cells and progenitor cells.
- Immune-mediated destruction.
- Primary stem cell abnormality—**inherited defect** in the stem cells.

Clinical Features

- Any age of both sexes
- Insidious
 - Progressive weakness, pallor and dyspnea due to anemia.
 - Frequent (mucocutaneous bacterial infections) or fatal infections due to neutropenia.
 - Bleeding manifestations in the form of petechiae, bruises and ecchymoses due to thrombocytopenia.

BOX 10.4: Common causes of aplastic anemia**1. Acquired****Idiopathic**

- Acquired defects in stem cell
- Immune mediated

Secondary**Chemical Agents**

- Cytotoxic drugs: Alkylating agents, antimetabolites
- Inorganic arsenicals

Idiosyncratic

- Chloramphenicol
- Penicillamine
- Gold salts
- Methylphenylethyl hydantoin

Physical Agents: Whole-body irradiation

Viral Infections: Hepatitis virus, Epstein-Barr virus, cytomegalovirus, herpes zoster (Varicella-zoster), HIV

- Benzene
- Chloramphenicol

- Phenylbutazone
- Carbamazepine
- Organic arsenicals

6 "I"s of the causes of aplastic anemia:

1. Idiopathic
2. Ingestion of drugs and chemicals
3. Idiosyncratic
4. Irradiation
5. Infections and
6. Inherited.

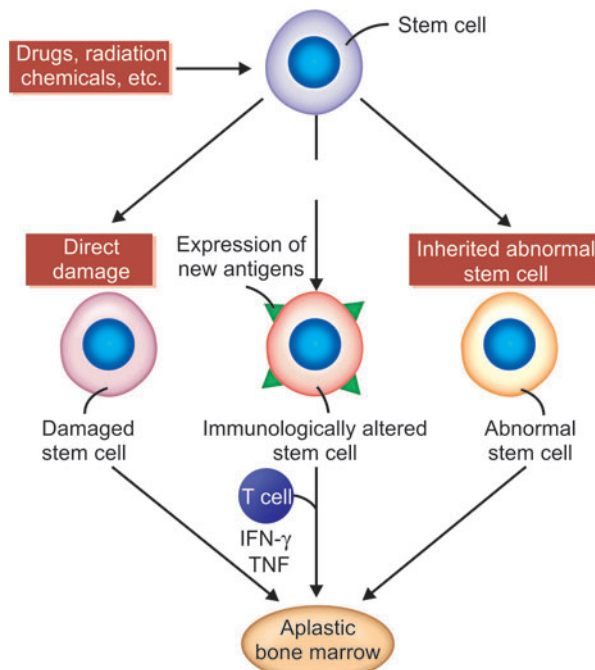
2. Inherited: Fanconi anemia, telomerase defects

Fig. 10.9: Pathogenesis of aplastic anemia

Q. Write short note on peripheral smear in aplastic anemia.

- **Peripheral smear: Pancytopenia**, i.e. decreased red cells, neutrophils and platelets.
 - **RBCs:** Normocytic normochromic anemia
 - **WBCs:** Total leukocyte count decreased. **Neutrophils markedly diminished** and neutropenia is a reflection of the severity of aplasia. Initial stages, lymphocytes normal in number as the disease progresses their count decreases.
 - **Platelets:** Count is decreased.

BONE MARROW

Bone marrow elements are replaced by fat and aspiration usually yields dry tap.

- **Marrow aplasia**—best appreciated in a **bone marrow (trephine) biopsy**
 - **Cellularity:** Marked **hypocellularity**.
 - **Hematopoiesis:** **Paucity of all erythroid, myeloid and megakaryocytic precursors**.
 - **Other cells:** **Lymphocytes and plasma cells are prominent**.

Reticulocyte count is markedly low in aplastic anemia and is characteristic feature.

Laboratory Findings**Peripheral Blood**

- **Hemoglobin**
- **Packed cell volume (PCV)**
- **Reticulocyte count:** Markedly decreased.

No Splenomegaly

Absence of splenomegaly and in its presence the diagnosis of aplastic anemia should not be made.

Diagnosis: Diagnosis is made with **peripheral blood and bone marrow biopsy findings**.

Differential Diagnosis

Q. Write short note on pancytopenia.

- Should be distinguished from other causes of pancytopenia (Box 10.5).

BOX 10.5: Causes of pancytopenia

Decreased bone marrow function

- Aplastic anemia
 - Idiopathic
 - Secondary
 - Inherited
- Myelodysplastic syndromes
- Bone marrow infiltration with:
 - Leukemia
 - Lymphoma
 - Myeloma
 - Tumors (carcinoma)
 - Granulomatous diseases (e.g. tuberculosis, sarcoidosis)
- Nutritional deficiencies:
 - Megaloblastic anemia (vitamin B₁₂ and folic acid deficiency)
- Paroxysmal nocturnal hemoglobinuria
- Myelofibrosis (rare)
- Hemophagocytic syndrome

Increased peripheral destruction

- Hypersplenism

Prognosis: Unpredictable.

HEMOLYTIC ANEMIAS DUE TO RED CELL MEMBRANE AND ENZYME DEFECTS

HEMOLYTIC ANEMIA

Q. Define and enumerate the causes/classify hemolytic anemia.

Definition

Hemolytic anemias are due to **increase in the rate of red cell destruction** (hemolysis).

Normal lifespan of red cell is about 120 days. In hemolytic anemias RBC survival time is considerably shortened.

Classification of Hemolytic Anemias (Table 10.9)

Breakdown of normal RBCs occurs in the macrophages of the bone marrow, liver and spleen.

Depending on:

- **Location of hemolysis:** Intravascular and extravascular
- **Source of defect causing hemolysis:** Intracorporeal defect and extracorporeal defect
- **Mode of onset:** Hereditary and acquired disorders.
- **Underlying mechanisms of hemolysis** (Box 10.6).

BOX 10.6: Classification and causes of hemolytic anemia

Inherited Genetic Defects

- **Red cell membrane disorders:** Hereditary spherocytosis, hereditary elliptocytosis
- **Enzyme deficiencies**
 - **Hexose monophosphate shunt enzyme deficiencies:** G6PD deficiency
 - **Glycolytic enzyme deficiencies:** Pyruvate kinase deficiency, hexokinase deficiency

Hemoglobin Abnormalities

- **Deficient globin synthesis:** Thalassemia syndromes
- **Structurally abnormal globins (hemoglobinopathies):** Sickle cell disease

Acquired Genetic Defects

- **Deficiency of phosphatidylinositol-linked glycoproteins:** Paroxysmal nocturnal hemoglobinuria

Antibody-Mediated Destruction (immuno-hemolytic anemias)

- **Isohemagglutinin:** Hemolytic disease (Rh disease) of the newborn, transfusion reactions
- **Autoantibodies:** Idiopathic (primary), drug-associated, systemic lupus erythematosus

Mechanical Trauma to RBCs (Fragmentation syndrome)

- **Microangiopathic hemolytic anemias:** Hemolytic uremic syndrome, disseminated intravascular coagulation, thrombotic thrombocytopenia purpura
- **Cardiac traumatic hemolysis:** Defective cardiac valves

Infections of Red Cells: Malaria, babesiosis

Toxic or Chemical Injury: Clostridial sepsis, snake venom, lead poisoning

Sequestration: Hypersplenism

Abbreviations: G6PD, glucose-6-phosphate dehydrogenase; PK, pyruvate kinase

Decreased red cell survival does not always cause anemia as there is a compensatory increase in red cell production by the bone marrow.

Location of Hemolysis

It may be intravascular and/or extravascular. The differences between these two types are listed in Table 10.4.

TABLE 10.4: Differences between extravascular and intravascular hemolysis**Q. List the differences between extravascular hemolysis and intravascular hemolysis.**

Characteristics	Extravascular hemolysis	Intravascular hemolysis
Site of hemolysis	RE system (spleen, bone marrow)	Within circulation
Splenomegaly	Usual	Uncommon
Laboratory findings <ul style="list-style-type: none"> • Serum bilirubin-unconjugated • Serum haptoglobin • Hemoglobinemia 	Moderately raised Normal Not seen	Mildly raised Decreased Positive
Urine <ul style="list-style-type: none"> • Hemoglobinuria • Hemosiderinuria 	Absent Absent	Present Present
Examples	Thalassemia, sickle cell anemia	G6PD deficiency, PNH

Abbreviations: G6PD, glucose-6-phosphate dehydrogenase; PNH, paroxysmal nocturnal hemoglobinuria

In most hemolytic anemias red cell destruction is extravascular.

Laboratory Findings in Hemolytic Anemias

Q. Write short essay/note on laboratory findings in hemolytic anemias.

Q. Write short essay/note on peripheral blood picture of hemolytic anemias.

Peripheral Blood

Hemoglobin: It is decreased and varies with the type and duration of hemolytic anemia.

Reticulocyte count: Increased.

Peripheral smear: It is the most important investigation in hemolytic anemia. The following findings alone or in combination suggest hemolysis:

- **Red blood cells (RBCs):** They show markedly increased **reticulocyte count**, which appear as large **polychromatophilic** red blood cells in the peripheral blood. Moderate to marked hemolysis results in appearance of **nucleated red cells**, mostly late normoblasts. Red cell morphology provides a clue to the underlying hemolytic disorder like: spherocyte, sickle cell, target cell, acanthocyte, schistocyte, malarial parasite, etc.
- **White blood cells:** **Neutrophilia** with shift to left (increase in the percentage of immature/young neutrophils in the circulating blood) and presence of metamyelocytes and myelocytes is seen in active hemolysis.
- **Platelets:** In acute hemolysis, there is **thrombocytosis** with numerous large platelets.

Bone Marrow

Bone marrow examination is usually not necessary for the diagnosis of hemolytic anemia.

- **Cellularity:** Due to erythroid hyperplasia, the overall cellularity of the bone marrow is increased.
- **Erythropoiesis:** Anemia with tissue hypoxia stimulates increased production of erythropoietin, which causes erythroid hyperplasia in bone marrow. Erythroid hyperplasia is the morphological hallmark of various hemolytic anemias and is characterized by **increased number of erythroid precursors (normoblasts) in the marrow**.
- **M:E ratio:** The myeloid-erythroid ratio is decreased with a reversal ranging from 1:1 to 1:6.

Extramedullary Hematopoiesis

- Extramedullary hematopoiesis develops when marrow erythroid hyperplasia is not able to ameliorate moderate to severe anemia because of **“ineffective erythropoiesis”**. It can appear in the liver, spleen and lymph nodes.
- Mostly found in hereditary hemolytic anemias like thalassemia and sickle cell anemia. X-ray of the bone show expansion of marrow space especially in tubular bones and skull.

Features of Increased Red Cell Destruction

- Increased unconjugated bilirubin in blood: **Jaundice**.
- **Increased stercobilinogen** in stool causing dark-colored stool.
- **Increased urobilinogen in urine** leading to **high-colored urine**.

- **Increased iron stores**-iron released from heme is stored in bone marrow.
- **Characteristic findings of anemia due to intravascular hemolysis**
 - Hemoglobinemia
 - **Decreased serum haptoglobin**: It is characteristic of intravascular hemolysis.
 - Hemoglobinuria
 - Hemosiderinuria
 - Plasma lactate dehydrogenase (LDH): Increased

Features of Increased Red Cell Production

- Anemia and resultant tissue hypoxia causes increased erythropoietin production by kidney.
- Peripheral blood (mentioned above).
- Bone marrow: Increased erythropoietin stimulates bone marrow and produces **compensatory erythroid hyperplasia**. Bone marrow hyperplasia leads to **increased reticulocytes** in the peripheral blood.

Features of Damaged Red cells

- Morphological features: These include presence of microspherocytes, elliptocytes, red cell fragments, etc. in the peripheral blood.
- Lifespan of red cells: Red cell survival is shortened and can be detected by 51Cr labeled method.
- Other tests include: Osmotic fragility test, autohemolysis test, antiglobulin tests, electrophoresis for abnormal hemoglobins, estimation of HbA₂, HbF, sickling test and screening test for G6PD deficiency.

Laboratory features of hemolytic anemia are summarized in Box 10.7.

HEREDITARY SPHEROCYTOSIS

Hereditary spherocytosis (HS) is a rare **inherited hemolytic anemia** resulting from the **defect in the red cell membrane**.

Normal structure of RBC membrane is depicted in Figure 10.10.

Etiopathogenesis

Q. Describe the etiopathogenesis/molecular pathology of hereditary spherocytosis.

- Autosomal dominant disorder
- RBC membrane protein defect caused by various mutations. Most common mutations involve **ankyrin, band 3, spectrin, or band protein 4.2**.

BOX 10.7: Summary of laboratory features of hemolytic anemia

1. **Features/consequences of increased RBC destruction/breakdown**
 - Anemia
 - Unconjugated hyperbilirubinemia (jaundice)
 - Increased urobilinogen in urine (resulting from bilirubin breakdown in the intestine) leading to high colored urine
 - Shortened red cell life span (demonstrated by 51 Cr-labeled red blood cells).
 - Other features:
 - In extravascular hemolysis: Splenomegaly usual
 - In intravascular hemolysis:
 - ◆ Decreased plasma haptoglobin and hemopexin
 - ◆ Increased plasma lactic dehydrogenase (LDH), hemoglobinemia, hemoglobinuria, hemosiderinuria and methemoglobinemia (in some)
2. **Consequences of increased RBC production**
 - Peripheral smear: Reticulocytosis
 - Bone marrow: Erythroid hyperplasia
3. **Morphological features of damaged red cells**
 - Peripheral smear: For example, microspherocytes, elliptocytes, red cell fragments.

HS, is due to defect in the RBC membrane protein. The common mutations involve ankyrin, band 3, spectrin or band protein 4.2.

Mechanism of Hemolysis in HS (Fig. 10.11)

- Young HS RBCs are normal in shape. But as they age, they undergo **loss of membrane fragments in the circulation**. These small RBCs assume a **spherical shape (spherocytes)**.
- **Spherocytes are rigid, inflexible and less deformable**. They get trapped in the spleen leading to **premature destruction** of spherocytes.

HS: intrinsic defect of RBC membrane-extravascular hemolysis.

Laboratory Findings

Q. Write short notes on laboratory findings in hereditary spherocytosis.

Peripheral Blood

- **Hemoglobin**: Decreased and level depends on degree of hemolysis.
- **Red cell indices**:
 - MCV: **reduced** (normal 82–98 fL)
 - MCHC: **raised and >35 g/dL** (normal 31–36 g/dL).

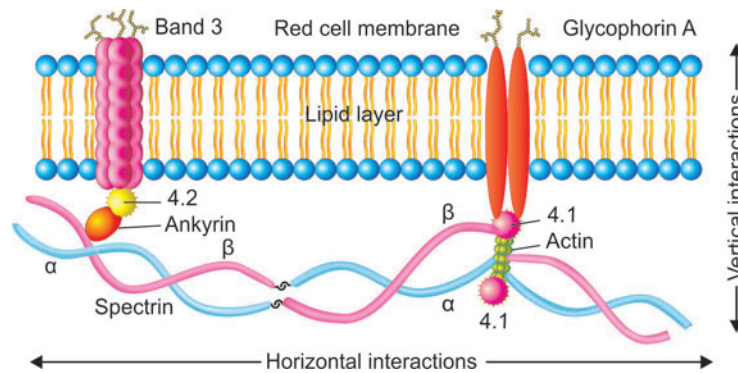


Fig. 10.10: Structure of the red cell membrane

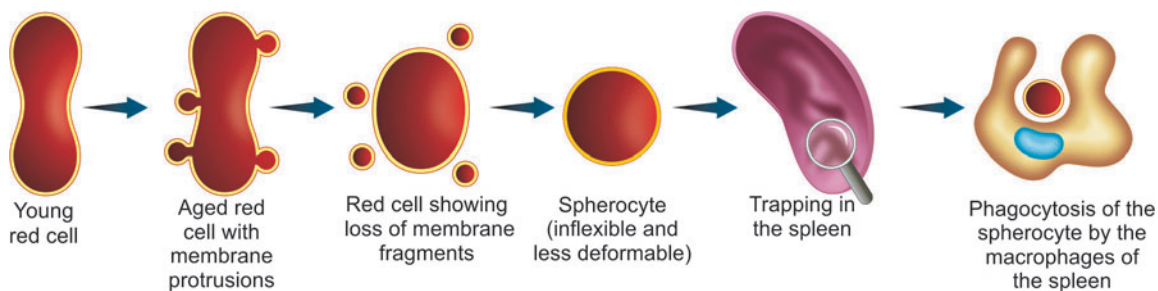


Fig. 10.11: Mechanism of hemolysis in hereditary spherocytosis

Q. Write short note on spherocyte.

- **Peripheral smear:** very important for diagnosis (Figs 10.12 and 10.13).
 - RBCs:
 - ♦ Spherocytes are most distinctive but not pathognomonic. Spherocytes are small, dark-staining (hyperchromic) RBCs without any central pallor.
 - ♦ Polychromatophilia due to reticulocytosis.
 - WBCs: Total leukocyte count (TLC) increased.
 - Platelets: Normal.

- **Reticulocyte count:** Increased (Fig. 10.14).

Q. Causes of spherocytes in peripheral smears.

In hereditary spherocytosis MCHC is >35 g/dL.

Spherocytes and reticulocytosis are observed in the peripheral blood.

Spherocytes are seen in hereditary spherocytosis. They may also be seen in autoimmune hemolytic anemia, ABO hemolytic disease of newborn, hypersplenism, hemolytic transfusion reaction and burns.

BONE MARROW

- **Cellularity:** Markedly hypercellular
- **Erythropoiesis:** Erythroid hyperplasia

- **Myelopoiesis:** Normal
- **Megakaryopoiesis:** Normal.

Bone marrow shows erythroid hyperplasia.

Autohemolysis Test

In this test blood is incubated at 37°C for 48 hours and amount of hemolysis noted. Hereditary spherocytes shows marked increase in spontaneous autohemolysis (10–15% of red cells compared to normal which is less than 4%) and may be partially corrected by addition of glucose.

Direct Antiglobulin (Coomb's) Test

It is negative and helps to distinguish from acquired immunohemolytic anemia where it is positive.

Biochemical Findings

- **Serum bilirubin:** mildly raised.
- **Urine urobilinogen:** increased.
- **Serum haptoglobin:** decreased.

Osmotic Fragility Test

Osmotic fragility is increased and there is shift of the curve to the right (Fig. 10.15).

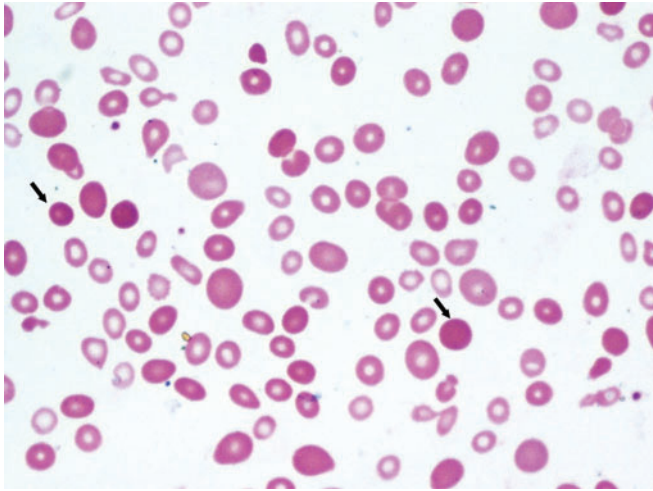


Fig. 10.12: Peripheral blood smear with numerous spherocytes (arrows)

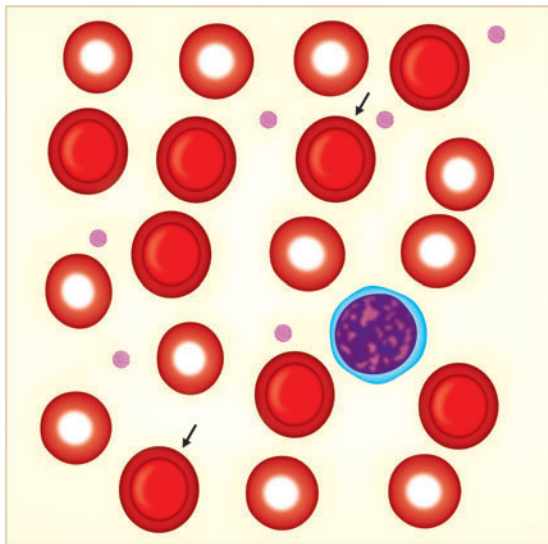


Fig. 10.13: Diagrammatic peripheral blood smear with numerous spherocytes (arrows)

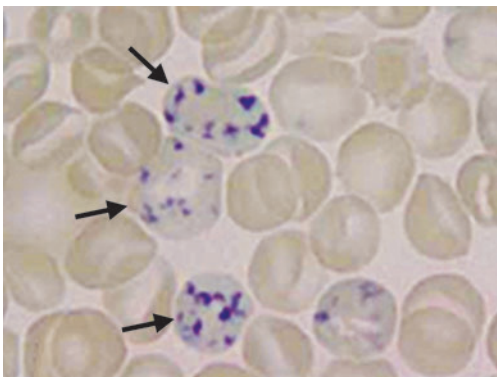


Fig. 10.14: Smear shows reticulocyte with blue filamentous/granular material (new methylene blue stain) (arrows)

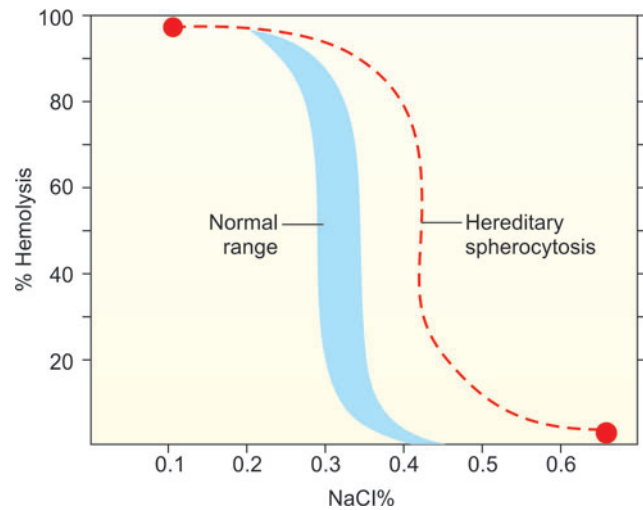


Fig. 10.15: Osmotic fragility test. Normal curve (blue) and increased osmotic fragility in hereditary spherocytosis

Clinical Features

- **Age:** Anytime from the **neonatal period to adulthood**.
- **Family history:** Most (75%) are inherited as **autosomal dominant trait**.
- **Anemia:** Mild to moderate.
- **Jaundice:** **Intermittent attacks**, precipitated by pregnancy, fatigue, or infection.
- **Splenomegaly:** **Moderate** (500 to 1000 g).
- **Gallstones:** **Pigment gallstones**.
- **Aplastic crises:** May be triggered by an acute **parvovirus** infection.

GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY

Clinical features of intermittent jaundice, splenomegaly and spherocytes in the peripheral smear is highly suggestive of HS.

Q. Write short note on G6PD deficiency.

- Hemolytic disease due to **red cell enzyme defects**.
- In G6PD deficiency, **RBCs are susceptible to oxidative injury** by free radicals.
- It is an **X-linked recessive disorder** and its **full expression is seen only in males**.
- There are different subtypes.

Role of G6PD (Fig. 10.16)

G6PD deficiency is an **intrinsic defect** and hemolysis is primarily **intravascular**.

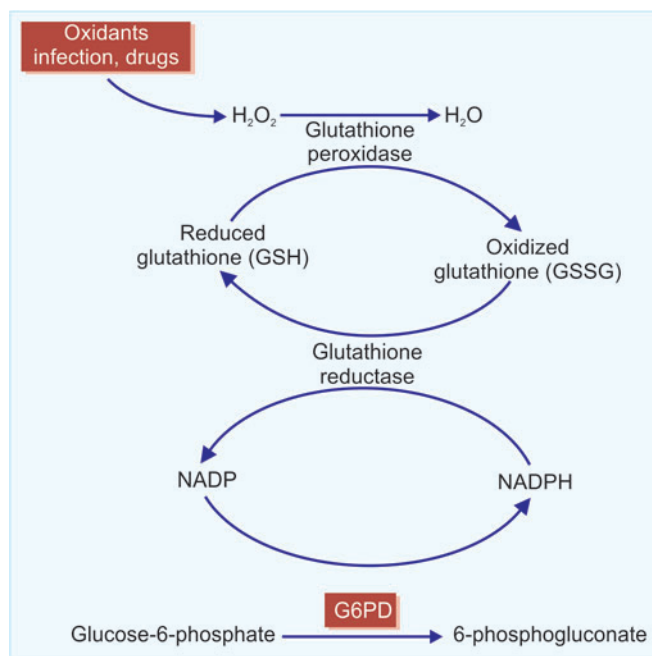


Fig. 10.16: Role of G6PD against injury by oxidants

- Reduced glutathione (GSH) in the normal RBCs protects them against oxidant injury by breakdown of compounds such as H_2O_2 to H_2O . The **housekeeping enzyme, G6PD** is required for normal GSH.

Sequence of Events in G6PD Deficiency

In G6PD deficiency, oxidants can cause **both intravascular and extravascular hemolysis**.

- In G6PD deficiency, there is decreased synthesis of **reduced glutathione**.
- RBCs when exposed to oxidant stress (during infections, exposure to drugs or chemical, fava beans) **accumulate H_2O_2** . It damages RBC membrane causing **hemolysis**.
- Hemolyzed red cells **liberate hemoglobin**.
- The **hemoglobin is oxidized** by oxidants leading to formation of **methemoglobin**, which forms **Heinz bodies** (Fig. 10.17) in the cytoplasm of RBCs.
- Heinz bodies removed from RBC membrane by macrophages in the spleen and produce **bite cells**. These bite cells are removed via **erythrophagocytosis in the spleen**.

In G6PD, RBCs exposed to oxidant stress, the hemoglobin is oxidized to methemoglobin which forms Heinz bodies in the cytoplasm of RBCs.

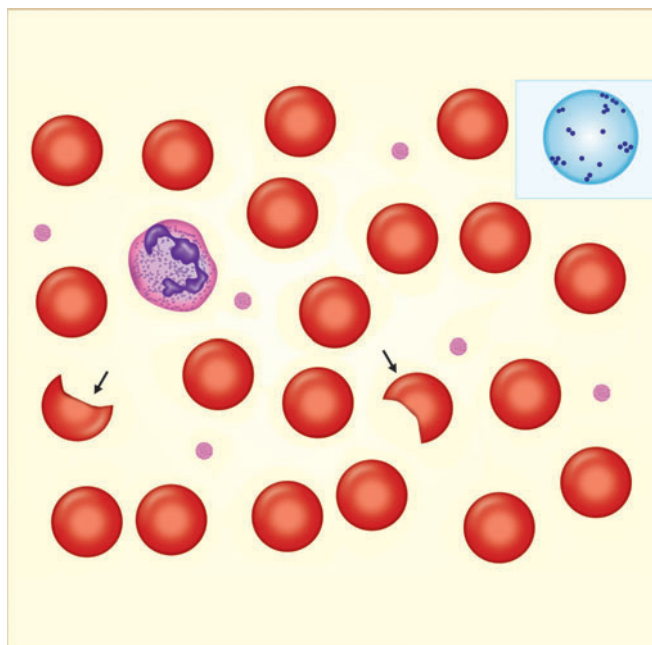


Fig. 10.17: Peripheral blood smear in G6PD deficiency with "bite cells" (arrows). Inset shows Heinz bodies (supravital stain)

G6PD deficiency has a protective effect against *Plasmodium falciparum* malaria.

Clinical Presentation

G6PD deficiency manifests in several distinct clinical patterns. Usually present as acute self-limited **acute intravascular hemolytic anemia** following exposure to oxidative stress.

Laboratory Findings

Peripheral Blood

- Hemoglobin: decreased.**
- Reticulocyte count: increased.**
- Peripheral smear:**
 - RBCs: Moderate anisopoikilocytosis with **polychromatophilia**, **microspherocytes** and **bite cells** (Fig. 10.17). **Heinz bodies** identified with a **supravital stain** and are best seen during active hemolysis.
 - WBCs: Mild leukocytosis.
 - Platelets: **Normal**.
- Self-limited hemolysis:** Primarily, the **old red cells are hemolyzed**, hence hemolysis is self-limited.

G6PD deficiency—oxidant damage to RBC

- Bite cells
- Heinz bodies.

Urine

Hemoglobinuria will be found during hemolysis and may last for about 1–6 days.

RBC Enzyme Analysis

Tests for G6PD deficiency are positive and should be assessed a few weeks after the acute hemolytic episode.

G6PD: enzyme analysis—confirmatory test.

THALASSEMIA SYNDROME

CLASSIFICATION OF HEREDITARY DEFECTS IN HEMOGLOBIN

Q. Classify hereditary disorders of hemoglobin.

Hemoglobin defects may be quantitative (reduced production of normal hemoglobin) or qualitative (production of abnormal hemoglobin).

- **Quantitative defect:** Genetic mutations in the globin loci (e.g. thalassemia) may quantitatively reduce the synthesis of α -globin or β -globin chain. It leads to net reduction of hemoglobin.
- **Qualitative defect:** Genetic mutations in the α -globin or β -globin locus may produce abnormal hemoglobin (e.g. sickle cell anemia). The abnormal hemoglobin may be functionally normal, but its physical or physiologic properties differ from normal hemoglobin.

The term hemoglobinopathy is usually used for a qualitative hereditary disorder of hemoglobin.

In α -Thalassemia, there is reduced/absence of synthesis of α -chains of globin.

THALASSEMIA SYNDROME

Q. Classify thalassemia syndromes.

- These are group of inherited disorders due to **abnormality of globin production**.
- It is characterized by **decreased or absence of synthesis of either α or β -globin chain of adult hemoglobin, HbA ($\alpha_2\beta_2$)**.

Classification

They are mainly classified as:

- **β -Thalassemia syndromes:** Impaired synthesis of β -chains of globin.
- **α -Thalassemia syndromes:** Impaired synthesis of α -chains of globin.
- **Miscellaneous thalassemia syndromes.**

In β -Thalassemia, there is decreased/absence of synthesis of β -chains.

β -Thalassemia

Q. Write in detail about β -thalassemia.

- **Autosomal recessive hereditary disorder**
- **Diminished synthesis of β -globin chains** and normal synthesis of α -chains.

Molecular Pathology

- β -globin chains are encoded by a single gene.
- The molecular errors over 200 genetic defects leading to β -thalassemia have been identified.
- Different types of **mutations in β -globin gene** can occur but mainly point mutations rather than gene deletions (unlike in α -thalassemia). The mutations result in defects in transcription, RNA splicing and modification, translation via frame shifts and nonsense codons. **Mutations leading to aberrant RNA splicing are the most common cause.**

Point mutations leading to aberrant RNA splicing is the most common cause of β -thalassemia.

Clinical and Genetic Classification (Table 10.5)

β -THALASSEMIA MAJOR

β -thalassemia is the commonest quantitative disorder of hemoglobin.

β -thalassemia major also called Mediterranean or Cooley's anemia.

- It is a **hereditary hemolytic anemia** due to **absence of synthesis of β -globin chain of hemoglobin**. The **synthesis of α -globin chain is not affected**.
- Homozygous form of β^0/β^0 or β^+/β^+ or double heterozygous β^0/β^+ (Box 10.7).

TABLE 10.5: Clinical and genetic classification of β -thalassemias

Clinical syndromes	Genotype	Clinical features
β -thalassemia major	Homozygous (β^0/β^0 , β^+/ β^+) or double heterozygous (β^0/β^+)	Severe form, severe anemia and transfusion dependent
		High level of HbF in the blood
β -thalassemia intermedia	Variable (β^0/β^+ , β^+/ β^+ , β^0/β , β^+/β)	Moderately severe and not transfusion dependent
β -thalassemia minor/ β -thalassemia trait	Heterozygous (β^0/β , β^+/β)	Mild anemia and asymptomatic

- β^0 = Total absence of β -globin synthesis
- β^+ = Markedly reduced or diminished β -globin synthesis
- β = Normal β -globin synthesis.

- Most common in Mediterranean countries, parts of Africa and South East Asia.
- Hemolytic anemia is of severe degree.

Pathophysiology of β -thalassemia Major (Fig. 10.18)

Q. Describe the pathophysiology/pathogenesis of β -thalassemia major.

Consequence of Defective or Absent β -chains

- **Severe hemolytic anemia** due to:
 1. **Absence of β -globin chain:** Results in **absence of synthesis of HbA** ($\alpha_2\beta_2$). This produces RBCs that are poorly hemoglobinized (hypochromic) and small in size (microcytic).
 2. **Ineffective erythropoiesis:** Unpaired and excess α -chains **aggregate** into insoluble precipitates, which bind to and **damage the membrane of erythroid precursors**. These erythroid precursors fail to mature and **undergo apoptosis** in the marrow.
 3. **Extravascular hemolysis:** RBCs with α -chain inclusions are removed by macrophages of spleen (extravascular hemolysis).
- **Synthesis of fetal hemoglobin (HbF):** The γ -globin chain synthesis continues even 6 months after birth and combines with α -globin leading to **increased levels of HbF** ($\alpha_2\gamma_2$). The level of HbF varies from 30–90%.

β -thalassemia major

- Absence of synthesis of HbA produces severe microcytic hypochromic anemia
- Increased synthesis of HbF.

Consequences of Ineffective Erythropoiesis

- **Changes in bone marrow:** Marked erythroid hyperplasia.
- **Changes in bone:**
 - **Skull X-ray:** Hair on end (“crew-cut”) appearance (Fig. 10.19)
 - **Typical facies:** **Thalassemic (chipmunk face) facies** (Fig. 10.20)—prominent forehead, cheekbones and upper jaw.
- **Extramedullary hematopoiesis:** in liver and spleen → consequent hepatosplenomegaly.
- **Cachexia:** Develops in untreated patients.

β -thalassemia major

- Thalassemic facies
- Crew cut appearance on skull x-ray
- Splenomegaly.

Iron Overload and its Consequences

- **Causes of iron overload:**
 1. **Increased absorption of dietary iron** from duodenum
 2. **Hemolysis**
 3. **Repeated transfusions** (usual mode of treatment).
- **Consequences:** Iron overload produces **hemosiderosis** and **secondary hemochromatosis** and damages to parenchyma of organs (e.g. heart, liver and pancreas).

β -thalassemia major:

- Iron overload damages parenchymal organs due to hemosiderosis and secondary hemochromatosis.

Clinical Features

- **Age:** Infants develop **moderate to severe anemia** 6–9 months after birth.
- **Growth and development:** Untreated/untransfused children **fail to thrive** and die within 4–5 years of age.
- **Bone changes:** Those who survive longer develop distortion of skull and facial bones. X-ray **skull** shows **hair on end appearance** (Fig. 10.19) and face shows a characteristic **thalassemic facies** (Fig. 10.20).
- **Marked splenomegaly:** Up to 1500 grams due to hyperplasia and extramedullary hematopoiesis.
- **Extramedullary hemopoiesis:** Liver and lymph nodes may show extramedullary hematopoiesis.

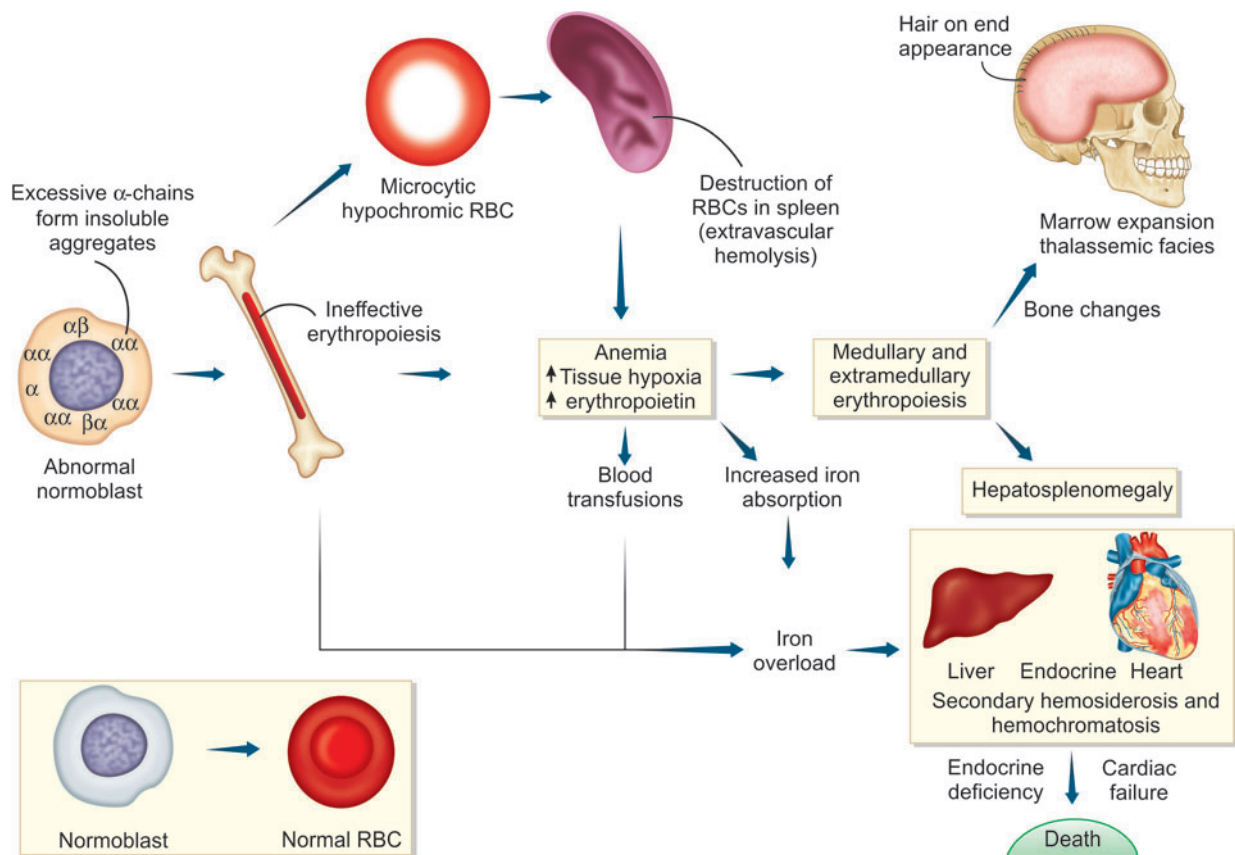


Fig. 10.18: Pathogenesis of β -thalassemia major and its consequence

- **Iron overload:** Multiple blood transfusions may lead to iron overload and result in **hemosiderosis and secondary hemochromatosis** (heart, liver and pancreas).

Failure to thrive, retarded growth, monogoloid face, and hepatosplenomegaly are clinical features of β -thalassemia major.

Laboratory Findings

Q. Mention the laboratory findings in β -thalassemia major.

Peripheral Blood

- **Hemoglobin** (ranges from 3–8 g/dL) and **hematocrit** (ranges from 8–23%): **Markedly reduced**
- **RBC count increased/normal** (in contrast to iron deficiency anemia).
- **Reticulocyte count increased** and in the range of 5–15%.
- **Red cell indices:**
 - **MCV decreased** and in the range of 45–70 fL (normal range 82–98 fL).
 - **MCHC decreased** and in the range of 22–30 g/dL (normal range 31–35 g/dL).
 - **MCH decreased** and in the range of 20–28 pg (normal range 27–32 pg).

β -thalassemia major: MCV, MCH and MCHC decreased.

• Peripheral smear:

Q. Write short note on peripheral smear findings in β -thalassemia major.

Q. Target cell and its appearance.

• RBCs:

- **Microcytic hypochromic anemia**
- Moderate to marked anisocytosis and poikilocytosis
- Many **target cells** (Figs 10.21 and 10.22). Target cell is morphologically abnormal RBC. In this hemoglobin is redistributed in such way that only the periphery and central region of RBC appear hemoglobinized and it resembles a target. These target cells are found in thalassemia major, sickle cell anemia, HbC, post-splenectomy, liver disease and obstructive jaundice
- Basophilic stippling
- **Nucleated red cell precursors** (normoblasts) in variable numbers (5–40%).

• WBCs: Leukocytosis with mild left shift.

• Platelets: Normal.

β -thalassemia major: The peripheral blood smear shows microcytic hypochromic anemia, target cells and anisopoikilocytosis.



Fig. 10.19: X-ray appearance of skull in β -thalassemia showing hair-on-end appearance (Courtesy: Dr Nuthan Kamath)



Fig. 10.20: Appearance of typical thalassemic facies (Courtesy: Dr Nuthan Kamath)

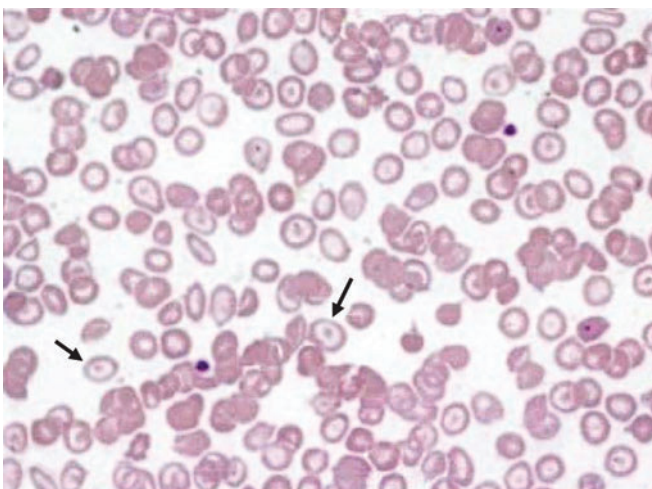


Fig. 10.21: Peripheral blood smear in β -thalassemia showing target cells (arrows)

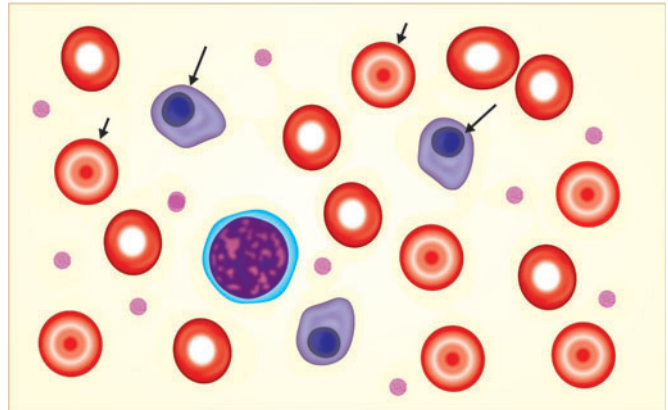


Fig. 10.22: Diagrammatic appearance of peripheral blood smear in β -thalassemia showing target cells (short arrows) and nucleated red cells (long arrows)

BONE MARROW

- **Cellularity:** Markedly hypercellular.
- **M:E ratio:** reversed to 1:1 to 1:5 depending upon the degree of erythroid hyperplasia.
- **Erythropoiesis:** Normoblastic with marked erythroid hyperplasia.
- **Myelopoiesis:** Normal.
- **Megakaryopoiesis:** Normal.
- **Bone marrow iron:** Markedly increased due to increased dietary absorption and hemolysis.

Bone marrow in β -thalassemia major shows marked normoblastic erythroid hyperplasia. Marrow iron is markedly increased.

Biochemical Findings

- **Bilirubin:** increased—mainly of unconjugated type.
- **Urine urobilinogen:** Increased
- **Serum haptoglobin:** Markedly reduced.
- **Serum iron status:**
 - Serum iron, serum ferritin and transferrin saturation are markedly increased
 - Total iron-binding capacity (TIBC): Reduced.

Special Tests

- **Fetal hemoglobin (HbF):** increased to 30–90% (normal range 0–1%).
- **Hemoglobin electrophoresis** (Table 10.6):
 - β^+ thalassemia (β^+/β^+ or β^0/β^+ genotypes): demonstrates bands of both HbA and HbF.
 - β^0 thalassemia (β^0/β^0 genotype): Since no β -chains are formed, there is no HbA. Major hemoglobin is HbF with normal or low HbA₂.

Reduced/absence of synthesis of β -chains; the excess α -chains combine with γ -chains leading to increased HbF.

- **High performance chromatography (HPLC):** HbF is increased (30–90%). HPLC measures various fractions of hemoglobin (Hb) and is used for **confirmation of diagnosis**.
- Prenatal diagnosis by **molecular analysis of DNA**.
- **Estimation of globin chains:** Normally α : β ratio is 1:1. Lack of β -chain alter this ratio to 5–30:1.

Differences between iron deficiency anemia and β -thalassemia major are presented in Table 10.7.

β -THALASSEMIA MINOR/TRAIT

- More common than β -thalassemia major.

TABLE 10.6: Hemoglobin F and A₂ percentage in thalassemia syndromes

Type	HbF	HbA ₂
β -Thalassemia major (homozygous)	30–90%	<3.5%
β -Thalassemia intermedia (double heterozygous)	10–30%	<3.5%
β -Thalassemia minor/trait (heterozygous)	0–5%	3.6–8%

Note: Normal adult cell contains 96% HbA ($\alpha_2\beta_2$), 3% HbA₂ ($\alpha_2\delta_2$) and 1% HbF ($\alpha_2\gamma_2$).

- Most patients are heterozygous for thalassemic gene.
- **Usually asymptomatic** and anemia is mild.

Laboratory Findings in β -Thalassemia Minor

- **Peripheral blood:** Microcytosis, hypochromia, basophilic stippling and target cells.
- **Bone marrow:** Mild erythroid hyperplasia.
- **Hemoglobin electrophoresis:** Increase in HbA₂ ($\alpha_2\delta_2$) to 4–8% of the total hemoglobin (normal $2.5 \pm 0.3\%$). HbF levels may be normal or slightly increased.
- **NESTROF test** (Naked eye single tube red cell osmotic fragility test): **positive**.
 - In this test, 0.02 mL of patient's blood is added to 5 mL of 0.35% saline in a test tube.
 - After half an hour white paper with a dark black line is held behind the tube.
 - The microcytic hypochromic RBCs of thalassemia minor are resistant to lysis than normocytic normochromic RBCs.
 - Hence, the black line on the paper is not clearly visible through the test tube compared to normal cells.
 - This test is used as screening test for thalassemia. However, this test is also positive in α -thalassemia trait, sickle cell trait and iron deficiency anemia.

TABLE 10.7: Differences between iron deficiency anemia and β -thalassemia major

Character	Iron deficiency anemia	β -thalassemia major
Etiology	Deficiency of iron	Reduced synthesis of β -chain
Laboratory findings		
• Peripheral smear		
– Type of RBCs	Microcytic hypochromic	Microcytic hypochromic
– Anisopoikilocytosis	Mild to moderate	Severe
– Target cells	Absent	Present
• Bone marrow iron	Absent	Markedly increased
• Serum iron profile		
– Serum ferritin	Reduced <15 $\mu\text{g/L}$	Increased (300–1000 $\mu\text{g/L}$)
– Serum iron	Reduced	Increased
– TIBC	Increased	Normal
• Fetal hemoglobin (HbF)	Normal (0–1%)	Markedly increased (30–90%)
Clinical features		
• Age	Any age	Presented <2 years of age
• Growth and development	Normal	Retarded
• Hepatosplenomegaly	Absent	Present
X-ray findings	Nil	Hair on end appearance

Abbreviations: RDW, red cell distribution width; TIBC, total iron-binding capacity.

β -thalassemia major should be differentiated from iron deficiency anemia. Treatment with iron in β -thalassemia major worsens the iron load and its consequences.

β -thalassemia intermedia: It is a clinical entity intermediate between thalassemia trait and thalassemia major.

β -thalassemia trait/minor should be differentiated from iron deficiency (Table 10.8).

TABLE 10.8: Differences between iron deficiency anemia and β -thalassemia minor/trait

Character	Iron deficiency anemia	β -thalassemia minor
Etiology	Deficiency of iron	Reduced synthesis of β -chain
Laboratory findings		
• Peripheral smear - RBCs	Microcytic hypochromic	Microcytic hypochromic
• Serum iron profile		
– Serum ferritin	Reduced <15 $\mu\text{g/L}$	Normal/slightly increased
– Serum iron	Reduced	Normal
– TIBC	Increased	Normal
– Transferrin saturation %	<15%	30–40%
• HbA₂ level	Normal or decreased ($2.5 \pm 0.3\%$)	Increased (4–8%)
• RBC count	<5 million/cu mm	>5 million/cu mm
• RDW	Increased	Normal

- **Estimation of HbA₂:** HPLC is used for accurate estimation. HbA₂ estimation is diagnostic and level ranges from 4–8%.

NESTROF test positive because the microcytic hypochromic RBCs of β -thalassemia minor are resistant to lysis than normocytic normochromic RBCs.

α -THALASSEMIA

α -Thalassemia: Anemia due to—

- Lack of adequate hemoglobin
- Effect of excess unpaired non- α -chains (β , γ , δ).
- Inherited disorders characterized by **reduced or absent synthesis of α -globin chains**.
- Autosomal recessive disorder.

Molecular Pathology

In contrast to a single gene coding β -globin chain, each α -globin chain are encoded by two genes. **Deletion of α -gene is the most common cause** of reduced α -chain synthesis.

Clinical Syndromes

Four genes control α -chain synthesis. Severity of α -thalassemia varies greatly depending on the number of α -globin genes deleted (Table 10.9). Each of the four α -globin genes normally contributes 25% of the total α -globin chains.

TABLE 10.9: Clinical syndromes associated with α -thalassemia disorders

Clinical syndrome	No. of α -globin deleted	Clinicopathological features
Silent carrier state	1	Asymptomatic
α-Thalassemia trait	2	Usually asymptomatic. Normal hemoglobin level or minimal anemia
Hemoglobin H disease	3	Moderate microcytic hypochromic anemia
Hydrops fetalis (Hb Barts)	4	Severe form, fatal and usually results in intrauterine death

α -thalassemia is one of the cause of non-immune hydrops fetalis.

Immune hydrops fetalis is a hemolytic disease caused by blood group incompatibility between mother and fetus.

SICKLE CELL DISEASE

Sickle cell diseases are hemoglobinopathies characterized by qualitative defect in hemoglobin synthesis.

Definition: Sickle cell disease (SCD) is a group of **hereditary disorders** of hemoglobin characterized by **production of defective hemoglobin called sickle hemoglobin (HbS)**. On low oxygen tension or deoxygenation, HbS imparts **sickle shape to RBCs**. HbS is produced due to **qualitative defect** in hemoglobin production caused by **mutation in β -globin gene**.

TABLE 10.10: Classification of sickle cell disease

<i>Sickle cell anemia (SS)</i>	<i>Sickle cell trait (AS)</i>
<ul style="list-style-type: none"> Homozygous state—both the β-globin chains are abnormal/defective 	<ul style="list-style-type: none"> Heterozygous state—one gene is defective (for HbS) and while the other gene is normal (for HbA)
<i>Other sickling syndromes (Compound heterozygous)</i>	
<ul style="list-style-type: none"> If both the β-globin chains have different abnormalities, (e.g. Hb SC, Hb S-β-thalassemia)—termed as compound heterozygous 	

Classification of sickle cell disease is presented in Table 10.10.

Sickle cell anemia is a homozygous state in which both β -globin chains are abnormal.

SICKLE CELL ANEMIA

Sickle cell anemia: Autosomal recessive disorder with extravascular hemolysis.

Characteristic Features

- Autosomal recessive disorder manifests early in life.
- Homozygous state (SS) caused by a mutation in the β -globin gene.
- HbS constitutes more than 70% of hemoglobin in their RBCs with no HbA.

HbS provides protection against falciparum malaria.

Etiopathogenesis

Q. Discuss the etiopathogenesis of sickle cell anemia.

- Production of abnormal hemoglobin called sickle hemoglobin (HbS).
- Missense point mutation:** In HbS, there is substitution of glutamic acid by valine in the 6th position, the β -globin chain of hemoglobin (Fig. 10.23). It alters the solubility or stability of the hemoglobin and produces hemolytic anemia.
- HbS is responsible for the characteristics of the disease.

Sickle cell trait: one β -globin chain is abnormal and other β -globin chain is normal.

Replacement of the glutamic acid residue by valine in 6th position of β -globin chain.

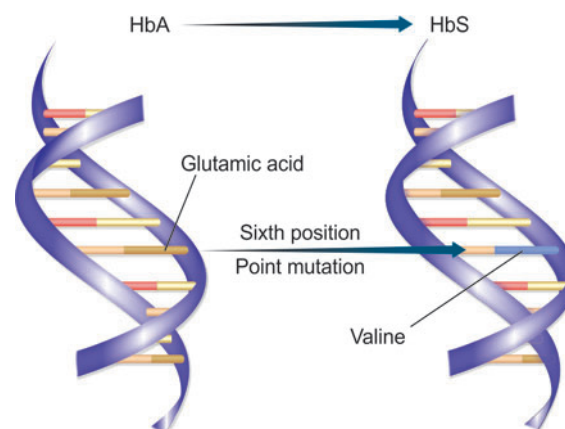


Fig. 10.23: Replacement of glutamic acid with valine in the sixth position of β -globin

Molecular Basis of Sickling (Fig. 10.24)

Q. Write short answer on irreversible sickle cells.

- During low O_2 tension or deoxygenation, HbS molecules undergo aggregation and polymerization.
- If deoxygenation continues, the aggregated HbS molecules form long needle-like fibers (or pseudocrystalline structures known as **tactoids**) within RBCs.
- The **tactoids** grow in length beyond the diameter of RBCs and distort RBC shape.
- RBC become elongated and assumes a shape like sickle (or crescent moon or holly-leaf or boat) and predisposes to stasis and vascular occlusion.
- When the oxygen tension returns to normal, the sickled red cell returns to normal shape.
- Recurrent sickling causes red cell membrane damage and these RBCs become **irreversibly sickled cells (ISC)**.

With repeated sickling the RBCs become irreversibly sickled cells (ISC) and leads to RBC membrane damage and hemolysis.

Factors Affecting Sickling (Table 10.10)

Mechanism of Red Cell Damage

- HbS polymerization:** When HbS polymerizes, it grows beyond the RBC membrane and project through it.
- Dehydration:** Repeated episodes of sickling leads to increased dehydration of RBCs. These RBCs become **more rigid and nondeformable** (irreversible sickled cells).
- Percentage of ISC:** Degree of the hemolysis correlates with the percentage of irreversibly sickled cells.
- Impaired cation homeostasis:** Structural changes in the RBC membrane causes the influx of Ca^{2+} ions, which activate an ion channel resulting in the efflux of K^+ and H_2O .

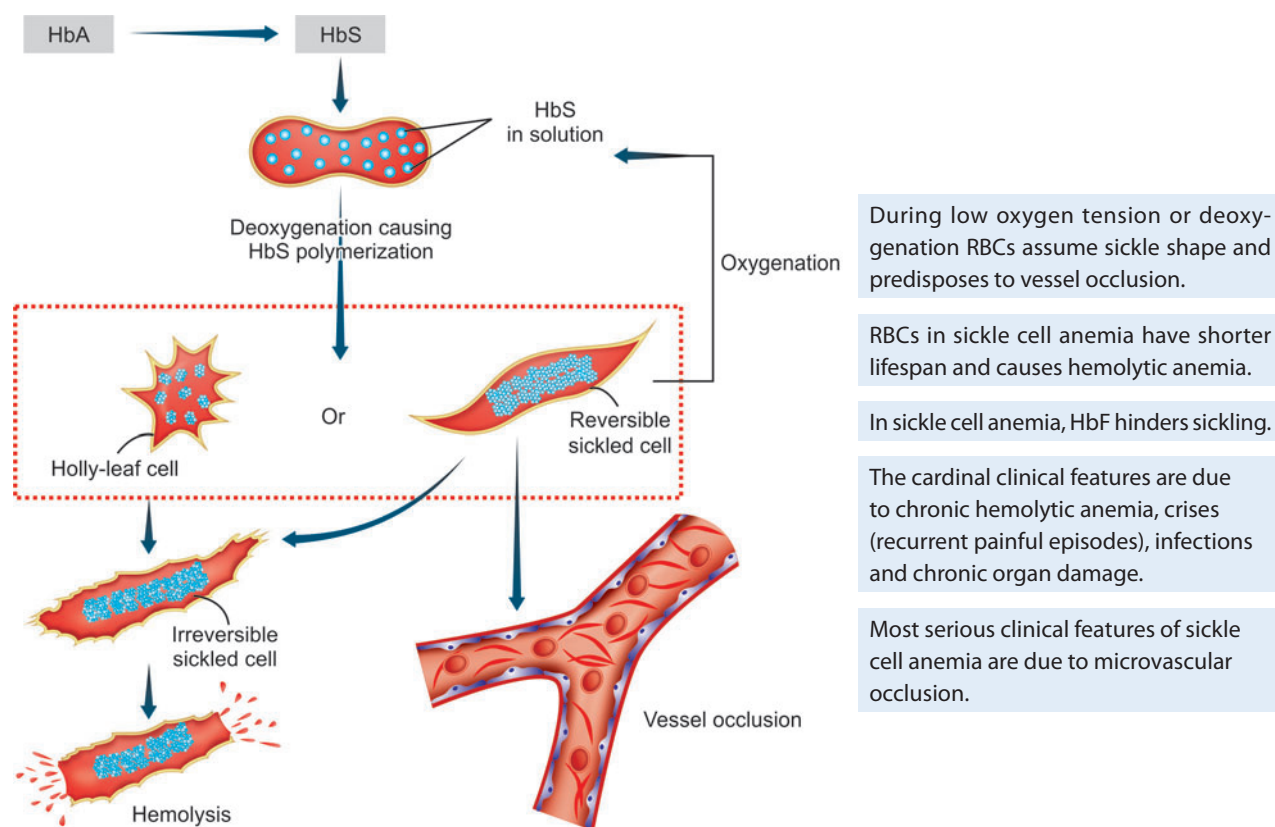


Fig. 10.24: Pathogenesis of sickle cell anemia

TABLE 10.11: Factors affecting sickling

Factors	Favors sickling	Hinders sickling
Type of other associated hemoglobins	-	HbA
	-	HbF
	HbC	-
Transit time in microvasculature	Slowing of bloodstream	-
MCHC	Increased MCHC	Decreased MCHC
Intracellular pH	Decreased pH	-
Other factors	Temperature above 37°C	-
	Infections	-

Abbreviation: MCHC, mean corpuscular hemoglobin concentration.

Pathogenesis of the Microvascular Occlusions

Most serious clinical features are due to occlusion of microvasculature.

- **Deformability:** Sick cells are rigid and tend to aggregate. The aggregated sickle cells block the small blood vessels.

- **Factors that slow the blood flow:** RBC cytoskeletal damage slow the movement of RBCs through microvascular beds.
- **Higher expression of adhesion molecules:** Sick cells express higher levels of adhesion molecules and thus become abnormally sticky to the endothelium.
- **Inactivation of nitric oxide:** Lysed sickle cells liberate free hemoglobin, which binds and inactivates nitric oxide (NO). This narrows the vessels and produces microvascular stasis and sickling.

Clinical Features (Fig. 10.25)

- Presence of **HbF** in the first 6 months of life has a protective role.
- **Symptoms appear after 6 months** of age as the HbF disappears.
- Infants and children present with acute problems like severe infection, acute chest syndrome, splenic sequestration and stroke.
- Chronic hypoxia in children is responsible for generalized impairment of growth and development. Adults manifest with chronic organ damage.

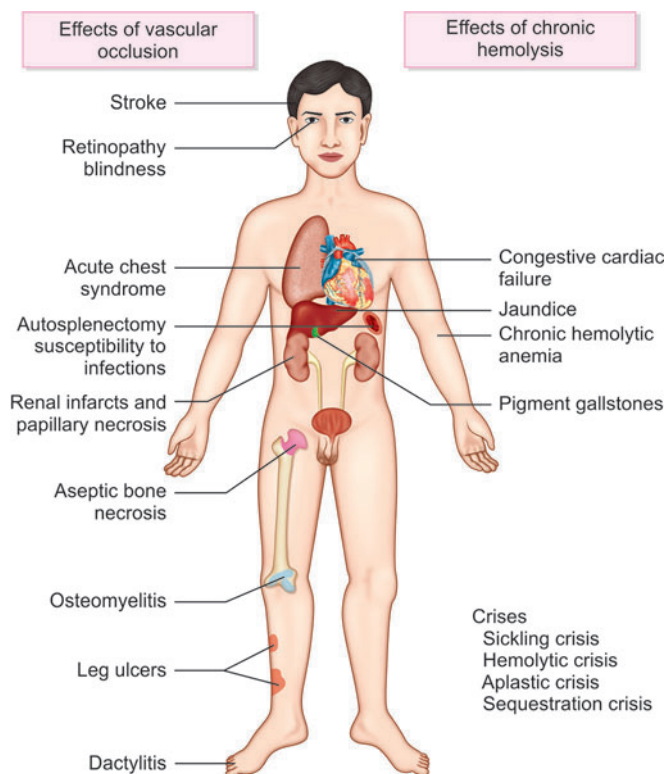


Fig. 10.25: Various effects of vascular occlusion and hemolysis in sickle cell anemia

Infants most commonly present with dactylitis.

Most common cause of death in adults is acute chest syndrome.

Chronic Hemolytic Anemia

Q. Complications of sickle cell anemia.

- Lifelong hemolysis (mainly extravascular) and causes chronic hemolytic anemia, which is of moderate degree. This produces raised unconjugated (indirect) bilirubin, and predisposes to pigment bilirubin gallstones (cholelithiasis) and cholecystitis.

Crises

Q. Write short essay/note on crises in sickle cell anemia.

Four crises encountered in sickle cell anemia: Sickling crisis, hemolytic crisis, aplastic crisis and sequestration crisis.

Four types of crises are encountered. These are:

1. Sickling Crisis (Vaso-occlusive/Pain/Painful/Infarctive Crisis)

- Most common
- Blockage of microcirculation by sickled red cells produces hypoxic injury and infarction.

- Bone: Manifest as the hand-foot syndrome, dactylitis of the bones of the hands or feet or both.
- Lung: Acute chest syndrome (dangerous).
- Spleen: Acute abdominal pain due to infarcts of abdominal viscera caused by occlusion of vessels. Recurrent splenic infarction results in autosplenectomy.

Recurrent splenic infarction due to sickling crisis lead to autosplenectomy.

2. Hemolytic Crisis

- Rare type and presents with marked increase in hemolysis.

3. Aplastic Crisis

- Associated with parvovirus B19.
- Reticulocytopenia.

4. Sequestration Crisis

- Usually occurs in **children**.
- **Sudden trapping** of blood in spleen or liver causes rapid enlargement of the organ and drop in hematocrit leading to hypovolemic shock.

Other crises encountered rarely are hypoplastic crisis and megaloblastic crisis (due to inadequate folate).

Reticulocytopenia is seen in aplastic crisis and reticulocytosis in sequestration crisis.

Increased Susceptibility to Infections

Susceptible to acute infections with encapsulated organisms.

- Common infections are pneumonia due to *Pneumococcus*, meningitis due to *Streptococcus pneumoniae* and osteomyelitis due to *Salmonella*. Increased frequency of osteomyelitis is due to bone infarcts, which act as a nidus for infection.
- Septicemia and meningitis are the most common causes of death in children.

Causes of Susceptibility to Infections

Common pathogens: *S. pneumonia*, *Salmonella* and *Pneumococcus*.

- Hypofunction of spleen:
In children: due to congestion and poor blood flow.
In adults: due to **multiple infarcts** and resultant **autosplenectomy**.
- Defects in the alternative complement pathway.
Impairs opsonization of encapsulated bacteria such as pneumococci and *Haemophilus influenzae*.

Chronic Organ Damage

SCA: severe hemolytic anemia; Sickling crisis; autosplenectomy.

Particularly seen in the spleen, bones, kidneys, heart, lungs, brain and skin.

- **Spleen**
 - Children after 6 months of life present with splenomegaly (up to 500 g).
 - After 5–6 years of age, the spleen gets fibrosed and **gradually reduces in the size** due to **multiple infarcts**.
 - Gradual **loss of splenic function** secondary to infarcts results in **autosplenectomy**.
- **Bone:** Osteomyelitis, particularly with *Salmonella typhimurium*.
- **Extremities:** Skin ulcers over the lower extremities.

Laboratory Findings in Sickle Cell Anemia

Q. Laboratory findings/diagnosis of sickle cell anemia.

Peripheral Blood

- Hemoglobin: Decreased.
- Hematocrit (PCV): Decreased.
- ESR: Reduced.
- Reticulocyte count: Increased and range from 3–10%.

• Peripheral smear

Q. Write short note on peripheral smear findings in sickle cell anemia.

- **RBCs:**
 - ◆ Normocytic normochromic to mildly hypochromic.
 - ◆ Moderate to severe degree of **anisopoikilocytosis**.
 - ◆ **Characteristic cell is the sickle cell**—appear as long, curved cells with pointed ends (Figs 10.26 and 10.27); may also show **target cells** (due to red cell dehydration) and **ovalocytes**.
 - ◆ **Polychromatophilia** due to reticulocytosis.
- **WBCs:** Mildly increased with shift to left.
- **Platelets:** Mildly increased.

Sickle cell anemia: ESR is reduced because sickle cells do not form rouleaux.

Peripheral smear shows characteristic sickle cells number of which varies.

BONE MARROW

- **Cellularity:** Hypercellular.
- **Erythropoiesis:** **Compensatory normoblastic erythroid hyperplasia**, which expands the marrow and causes resorption of bone and secondary new bone formation.

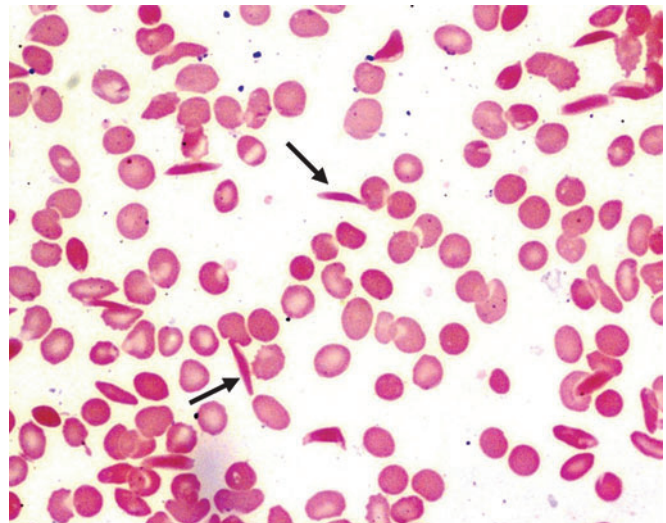


Fig. 10.26: Peripheral blood smear with sickle cells (arrows)

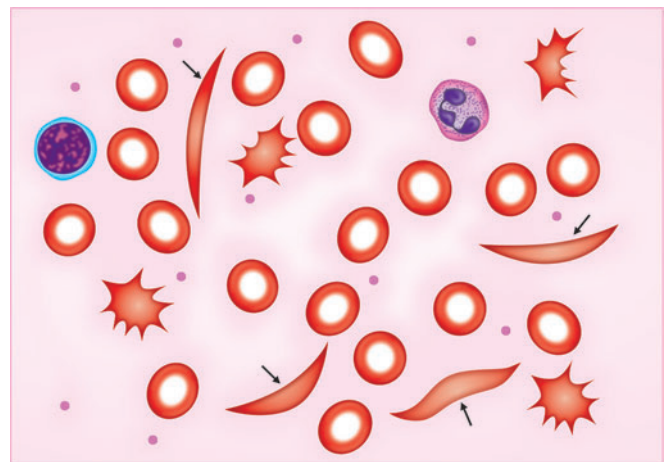


Fig. 10.27: Diagrammatic peripheral blood smear with sickle cells (arrows)

- **Myelopoiesis:** Normal.
- **Megakaryopoiesis:** Normal.
- **Iron stores:** Usually increased.

In severe cases, skull bone shows crew-cut appearance in roentgenograms.

Extramedullary hematopoiesis can also develop as a compensatory mechanism.

Serum Findings

- **Serum bilirubin:** Raised and predisposes to pigment gallstones.
- **Iron status:** Raised serum iron, serum ferritin and transferrin saturation.

- Serum haptoglobin: Reduced.
- Urine urobilinogen: Increased.

Diagnostic/Confirmatory Tests

Q. Enumerate tests of sickling in sickle cell anemia.

Q. Principle of sickling test.

Sickle cell anemia: HbS 70–90%, HbF 10–30%, no HbA.

- Sickling test:
 - **Principle:** Sickling is induced by adding a reducing (oxygen-consuming) agent like 2% sodium metabisulphite or sodium dithionite to blood sample.
 - Sickling is induced by adding a **reducing** (oxygen-consuming) **agent** like 2% sodium metabisulphite or sodium dithionite to blood sample.
 - Red cells with HbS show **sickled** (Fig. 10.28) and **holly leaf** appearance.
 - It is **diagnostic** of sickle cell anemia.
- **Solubility test for hemoglobin S:** This is performed by adding the anticoagulated blood to the reducing reagent solution (consisting of phosphate buffer, saponin and sodium dithionite). RBCs are hemolyzed and if HbS is present, it will be reduced by dithionite. HbS forms tactoids and refract light. The solution appears turbid whereas normal Hb gives a clear solution.
- **Hemoglobin electrophoresis:** HbS is a slow moving compared to HbA and HbF.
- **Estimation of HbF:** In homozygous state constitutes about 10–30% of hemoglobin.
- **HPLC:** Useful for confirmation of diagnosis.

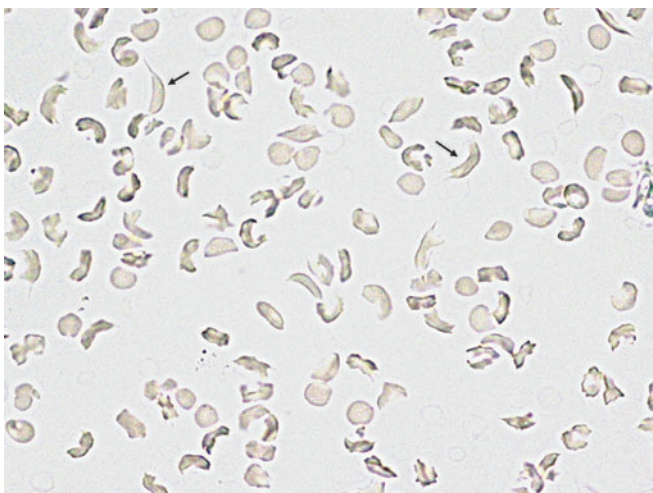


Fig. 10.28: Sickling test. Sickled red cells (arrows) induced by reducing agent (2% sodium metabisulphite)

Prevention of Sickle Cell Anemia

- **Prenatal diagnosis:** By analysis of fetal DNA obtained by amniocentesis or chorionic villous biopsy, to detect the point mutations.

Sickling test is a diagnostic test for sickle cell anemia.

SICKLE CELL TRAIT

Heterozygous state for the hemoglobin S mutation and shows both HbA and HbS (HbAS). One defective gene (from one parent with HbS) and while the other gene is normal.

Pathogenesis

In sickle cell trait, the hemoglobin A in RBCs prevents hemoglobin S polymerization. However, RBCs may sickle under extreme conditions (e.g. flight at high altitude in unpressurized aircraft, deep sea diving).

Clinical Features

Usually asymptomatic. Normal growth and development, lifespan and life expectancy.

Laboratory Findings

Peripheral Blood

- Hemoglobin: Normal or mildly decreased.
- Peripheral smear:
 - RBCs: Normocytic normochromic picture with very few target cells and mild degree of anisopoikilocytosis.
 - WBCs: Normal.
 - Platelets: Normal.

BONE MARROW

Hypercellular because of a compensatory normoblastic erythroid hyperplasia.

Sickle cell trait:

- Usually no anemia
- No significant clinical features
- Amount of HbS varies from 25% to 40%
- Hb A in RBCs prevents polymerization of Hb S.

Diagnostic Tests

In sickle cell trait: HbS 40–45% and HbA 55–60%.

- **Hb electrophoresis:** Demonstrates two bands of HbS and HbA.

- Sickling test: Sickling test is positive.
- High-performance liquid chromatography (HPLC): useful for confirmation of diagnosis.

Abnormal Hemoglobins

Q. List the abnormal hemoglobins.

These include HbS, HbC, HbD Punjab, HbE, Hb Barts, HbH, Hb Portland, HbO-Arab, etc.

OTHER ANEMIAS

IMMUNOHEMOLYTIC ANEMIAS

Anemias due to **premature RBC destruction** (hemolysis) mediated by **antibodies** that bind to RBCs. The antibodies may be either allo or auto type.

Immunoheolytic anemias are characterized by the destruction of RBCs by either allo or auto antibodies.

Classification of Immunoheolytic Anemias (Box 10.8)

Immunoheolytic anemias are mainly classified as:

1. Alloimmune and
2. Autoimmune hemolytic anemia.

Alloimmune Hemolytic Anemia

Hemolytic transfusion reactions are due to ABO mismatch. The antibodies present in the recipient's serum coat donor's RBCs and lead to intravascular hemolysis.

- Production of antibody against foreign antigen not present on individual's red blood cell.

BOX 10.8: Classification of immunoheolytic anemias

Alloimmune hemolytic anemia

- Hemolytic disease of the newborn
- Hemolytic transfusion reactions: Mismatched blood transfusion

Autoimmune hemolytic anemia

- **Warm antibody type (IgG antibodies active at 37°C)**
 - Primary (Idiopathic)
 - Secondary: Autoimmune disorders (systemic lupus erythematosus), drugs, lymphomas
- **Cold agglutinin type (IgM antibodies active at 4°C–18°C)**
 - Acute: mycoplasmal infection, infectious mononucleosis
 - Chronic: Idiopathic, lymphomas
- **Cold hemolysin type (Donath-Landsteiner antibodies)**

- Alloantibodies are present either in the serum or bound to red cells.

HEMOLYTIC DISEASE OF THE NEWBORN

Q. Write short notes on hemolytic disease of newborn.

- It is an alloimmune hemolytic anemia developing in the fetus and newborn baby.
- Hemolysis is extravascular.
- HDN develops when the IgG antibodies against blood group of fetus passes from mother to fetus through the placenta.
- Occurs in two forms:
 - **Rh incompatibility** in which mother is Rh negative and fetus is Rh positive. The anti-D antibodies are responsible for the hemolytic anemia.
 - **ABO incompatibility** in which mother's blood group is O and fetus is either of A or B blood group. Either anti-A or anti-B antibodies cause hemolysis.

HDN may be either due to Rh or ABO incompatibility between mother and fetal RBCs.

Rh Hemolytic Disease of the Newborn (Fig. 10.29)

Q. Write short note on Rh hemolytic disease of newborn.

Rh hemolytic disease of the newborn is more important than due to ABO incompatibility.

Pathogenesis

- Occurs when **mother is Rh (D antigen) negative and fetus is Rh positive.**
- **Sensitization** occurs when fetal Rh positive RBCs enter into Rh negative mothers. Rh negative mother develops **anti-Rh antibodies.**
- **Sensitization occurs** only at the time of **delivery or during miscarriage**. So, it **does not manifest in the first pregnancy.**
- In subsequent pregnancy, **anti-Rh antibodies from mother cross placenta and coat the Rh positive fetal red cells.** These antibodies cause immune **destruction of fetal red cells results in severe hemolytic anemia** leading to **jaundice** of the newborn.
- Fetus may develop cardiac failure—**hydrops fetalis** (immune type).

HDN usually does not manifest during first pregnancy. Sensitization develops during delivery or miscarriage.

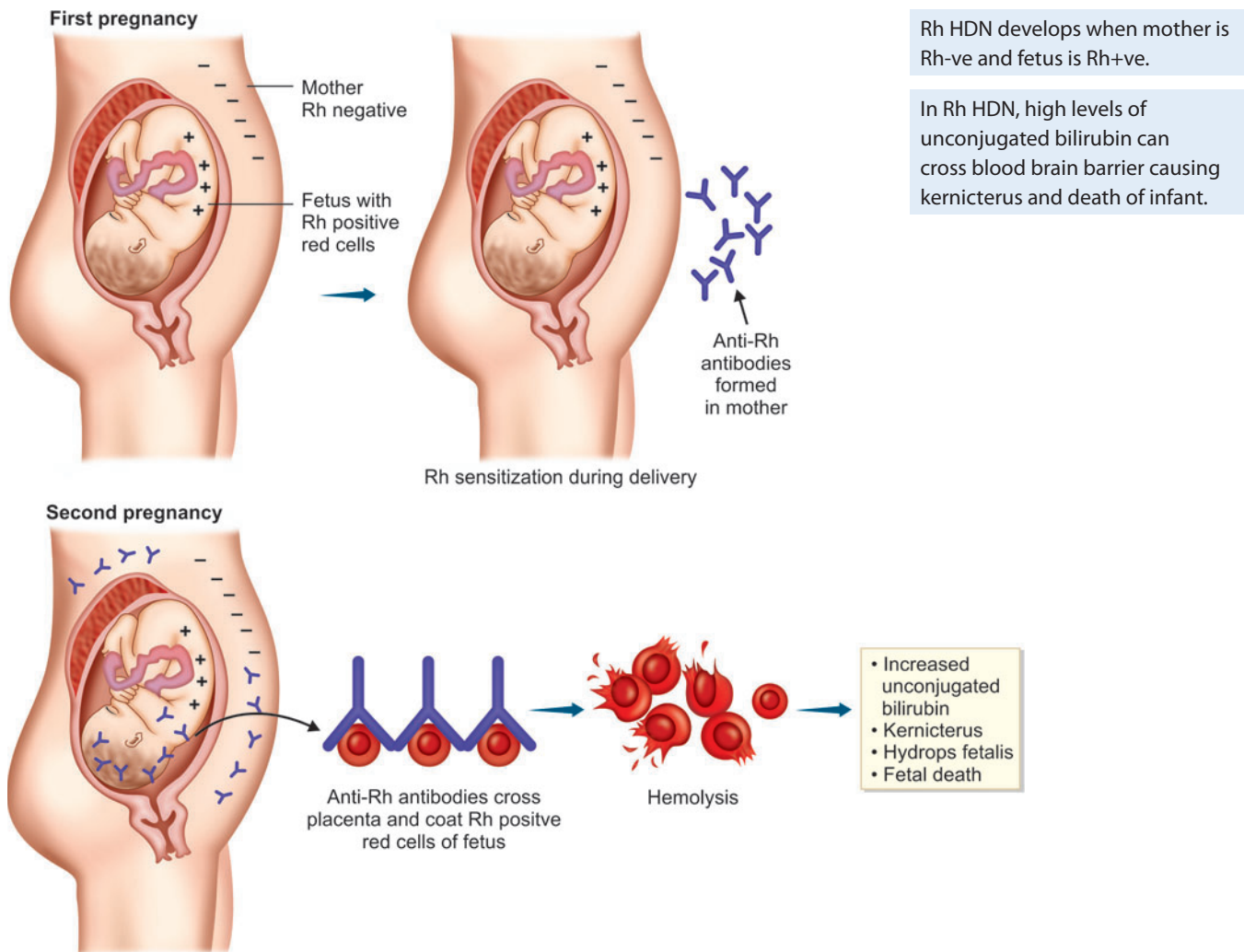


Fig. 10.29: Pathogenesis of Rh hemolytic disease of the newborn

Hydrops fetalis is fatal condition, characterized by left and right-sided heart failure producing generalized edema and may result in death.

Clinicopathological Features

- Infants may have jaundice at birth.
- When the disease is severe, the levels of **unconjugated bilirubin** in the blood are **high** and bilirubin can **pass the blood brain barrier**.
- Bilirubin is deposited in the **central nervous system** (especially the basal ganglia) producing **neurological damage** and is known as **kernicterus** (yellow coloration of cerebellum and basal ganglia due to bilirubin deposition). It can cause **death** of the infant.

Prevention of Rh HDN: By the prophylactic removal of fetal cells entering the maternal circulation before sensitization

develops, by **injecting anti-D** into the **Rh D negative mother**.

Laboratory Findings

Peripheral Blood

- Hemoglobin: decreased.
- Reticulocyte count: increased.

Peripheral smear:

- **RBCs:** Normocytic normochromic anemia with numerous nucleated RBCs, polychromatophils and occasional spherocytes.
- **WBCs:** Normal.
- **Platelet:** Normal.

Peripheral smear: Normocytic normochromic anemia with nucleated RBCs and polychromatophils.

- **Antiglobulin test (Coombs test):** Antibodies in the mother and baby are detected by indirect and direct Coombs test respectively (Fig. 10.30).

Serum Findings

- Serum bilirubin: Increased.
- Lactate hydrogenase (LDH): Increased.
- Haptoglobin: Decreased.

ABO Hemolytic Disease of the Newborn

ABO HDN is more common but less severe. It may be seen in first pregnancy.

- It is less severe.
- The fetus may be affected in the first pregnancy of a **mother with blood group O**.
- The IgG antibodies to A or B from maternal blood cross placenta and enter the fetal circulation. These **anti-A or anti-B antibodies** react with A and B antigenic determinants present in **fetal fluids and tissues**.
- This results in consumption of major portion of the maternal IgG and the small portion, which is left combines with fetal red cells causing **only mild hemolysis**.

ANTIGLOBULIN (COOMBS) TEST

Q. Write short notes on Coombs (antiglobulin) test.

It is useful to detect the presence of incomplete antibody (IgG) and/or complement on the RBC membrane.

Principle

- RBCs coated with incomplete antibody (IgG) or C3 complement does not cause agglutination of RBCs.
- Coombs reagent contains antibodies (antiglobulins) against human IgG/IgM/complement.
- If the RBCs coated by incomplete antibody or complement, are **treated with Coombs reagent**, the antiglobulins in the reagent will **induce agglutination** of such RBCs.

Types of Antiglobulin Test (Fig. 10.30)

There are 2 types of antiglobulin test: Direct and indirect.

- **Direct (Coombs) antiglobulin test (DAT)**
- **Indirect (Coombs) antiglobulin test (IAT)**

Direct Antiglobulin Test (Fig. 10.30)

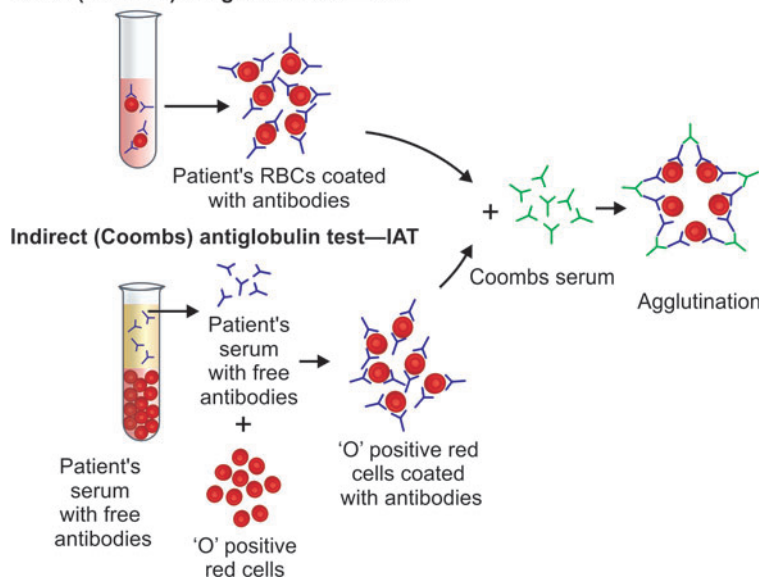
Q. Write short notes on Direct Coombs (antiglobulin) test, its procedure and conditions in which it is positive.

Patient's red cells are used in direct antiglobulin test.

Direct antiglobulin test (DAT) (direct Coombs test) **detects antibodies (IgG) and/or complement coated on the surface of patient's RBC membrane**.

- Patient's RBCs are taken in a test tube and washed three times in normal saline.
- Coombs (antiglobulin) reagent is added and observed for agglutination.

Direct (Coombs) antiglobulin test—DAT



In direct antiglobulin test, patient's RBCs are used where as in indirect antiglobulin test patient's serum is used for the test.

Antiglobulin test is useful for diagnosis of HDN.

Fig. 10.30: Direct and indirect methods of antiglobulin test (Coombs test)

- Agglutination indicates the presence of antibody on the RBC membrane and interprets as positive DAT.

Uses of Direct Antiglobulin Test

- **Hemolytic disease of the newborn (HDN)**, in which direct Coombs test is performed on the newborn baby's red cells from the cord blood. This test will be positive.
- **Autoimmune hemolytic anemia:** To demonstrate in vivo attachment of antibodies to red cells.
- **Drug-induced red cell sensitization.**
- **Investigation of hemolytic transfusion reaction.**

Indirect Antiglobulin Test (Fig. 10.30)

Patient's serum is used for indirect antiglobulin test.

Indirect antiglobulin test (IAT) (indirect Coombs test) **detects the presence of incomplete (IgG) antibodies and/or complement in the patient's serum.**

- In this test, patient's serum is taken and "O" Rh positive cell suspension of any normal individual is added.
- "O" Rh positive RBCs are coated with (IgG) anti-Rh antibodies (if present) in the patient's serum.
- Add Coombs (antiglobulin) reagent and examine for agglutination.
- Agglutination of RBCs indicates the presence of antibodies in the patient's serum and test is reported as positive for indirect antiglobulin test.

Patient's serum + O Rh positive RBC suspension + Coombs reagent → Agglutination (test positive).

Uses of Indirect Antiglobulin Test

- **Hemolytic disease of newborn:** Mother's serum is tested to detect anti-Rh antibody.
- **Cross-matching for blood transfusion:** To detect incompatibility of recipient's serum with donor's cells.

AUTOIMMUNE HEMOLYTIC ANEMIA

The type of antibody causing autoimmune hemolytic anemia may be warm antibody or cold agglutinin or cold hemolysin.

- Antibodies against self-antigens on the RBC membrane cause premature destruction of RBCs.
- Anti-RBC antibodies can be divided into three general categories (Box 10.8). Interaction of the autoantibody with the red cell antigen is **dependent on the temperature, i.e. warm or cold type.**

Warm Antibody Type

Warm AIHA: Mediated by IgG autoantibody-optimally active at 37°C.

- Most common type (50–70%).
- **Idiopathic** (primary) or **secondary** to drug exposure or predisposing disease.
- **IgG** type antibodies combine with RBC antigen at 37°C—**warm antibody.**
- **Direct antiglobulin test:** DAT (Coombs test) **positive** in 90–95% cases.
- **LE cell test:** **Positive** in SLE with secondary autoimmune hemolytic anemia (AIHA).

Cold Agglutinin Type

- Caused by **cold agglutinins.**
- Mediated by IgM antibodies optimally active below 30°C.
- Occurs as a complication of infections (e.g. infectious mononucleosis, *Mycoplasma* infections) and lymphoid neoplasms.

Cold Hemolysins Type (Donath-Landsteiner Antibodies)

- Autoantibodies directed against the P antigen system on red cells.
- Responsible for a rare disorder known as **paroxysmal cold hemoglobinuria.**
- **Direct antiglobulin test** is usually **negative.**

FRAGMENTATION SYNDROME

The RBCs subjected to **trauma** (physical or mechanical) in the circulation can undergo **fragmentation** and result in **intravascular hemolysis** leading to hemolytic anemias. These are known as fragmentation syndrome.

Classification

According to the site of hemolysis it is classified as:

- **Macroangiopathic** (large vessels) **hemolytic anemia:** They are also known as cardiac hemolytic anemias since the red cell trauma from an abnormal vascular surface (e.g. prosthetic heart valve, synthetic vascular graft).

Q. Write short note on microangiopathic hemolytic anemia.

- **Microangiopathic hemolytic anemia (MAHA):** it occurs in **capillaries** due to abnormal narrowing of the lumen (e.g. disseminated intravascular coagulation).

Laboratory Findings

Q. Write short note on peripheral blood picture in microangiopathic hemolytic anemia.

They are similar in both macro- and microangiopathic hemolytic anemias.

- **Hemoglobin:** Decreased. Anemia is mild to moderate.
- **Peripheral smear:**
 - RBCs: Show **fragmented red blood cells (known as schistocytes)**, **polychromasia**, with or without nucleated red blood cells and microspherocytes. The **fragmented red cells** assume different shapes like “**triangular cells**”, “**burr cells**”, “**helmet cells**” and are the **hallmark of diagnosis** of this group of hemolytic anemias.
- **WBCs:** Count within normal limits or increased.
- **Platelets:** Thrombocytopenia is often seen.

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

Q. Write short note on paroxysmal nocturnal hemoglobinuria.

PNH is an acquired disorder in which there is deficiency of GPI linked proteins, which normally protect the red cells against complement mediated lysis.

It is a **rare** and is the only hemolytic anemia **acquired** mutation in the hematopoietic stem cell.

Etiology and Pathogenesis

- **Acquired mutations in the phosphatidylinositol glycan-group A (PIGA) gene in the hematopoietic stem cell.**
- PIGA gene mutation causes **deficient synthesis of GPI-linked proteins** in blood cells and loss of anchor for decay-accelerating factor (DAF). Normally, DAF responsible for complement degradation.
- RBCs are abnormally sensitive to **complement-mediated intravascular hemolysis**.

In PNH, RBCs are very sensitive to complement-mediated hemolysis.

Clinical Features

- **Intravascular hemolysis:** Hemoglobin in acidic urine is converted into acid hematin and **results in dark brown urine**.
- **Thrombosis:** In the hepatic, portal or cerebral veins.

Laboratory Findings

PNH: Ham's acidified serum test and sucrose hemolysis test positive.

- **Ham's acidified serum test and sucrose hemolysis test:** Patient's RBCs undergo lysis when incubated with acidified serum (Ham test) or sugar (sucrose hemolysis test).
- **Flow cytometry:** Detects RBC deficient in GPI-linked proteins (CD55 and CD59) and is useful for diagnosis of PNH.

ANEMIAS OF BLOOD LOSS

During recovery phase of acute blood loss, peripheral smear show reticulocytosis.

Acute Blood Loss (Hemorrhage)

- Causes **loss of intravascular volume** and if massive can lead to **hypovolemic shock** and death.
- **Bleeding** may be **external** (e.g. open fracture, knife wound) or **internal** (e.g. ruptured spleen, ruptured abdominal aneurysm).

- **Peripheral smear:**
 - RBCs: **Normocytic normochromic** anemia. Polychromasia during the recovery phase due to **increased reticulocytes**.
 - WBCs: Leukocytosis.
 - Platelets: **Increased in number** (thrombocytosis) **during recovery phase**.

Chronic Blood Loss

Produces anemia when the rate of blood loss exceeds the regenerative capacity of the bone marrow or when iron reserves are depleted and results in **iron deficiency anemia**.

SIDEROBLASTIC ANEMIAS

Q. Write short note on sideroblastic anemia.

Sideroblastic anemias are rare refractory anemias which may be hereditary or acquired.

Rare heterogeneous group of refractory anemias characterized by:

- **Ringsideroblasts** in the bone marrow aspirate (Fig. 10.31).
- **Dimorphic peripheral blood picture:** Microcytic hypochromic red cells in hereditary form and macrocytic in the acquired forms of the disease mixed with normochromic cells.
- Iron-containing inclusions (**Pappenheimer bodies**) in the RBCs.

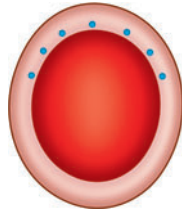


Fig. 10.31: Ring sideroblasts with partial perinuclear ring of iron granules

- Increased serum iron concentration and markedly increased storage iron.
- Ineffective erythropoiesis.

It is classified as:

1. Hereditary sideroblastic anemia
2. Acquired sideroblastic anemia: idiopathic or secondary.

CONTENTS OF BONE MARROW (BOX 10.9)

Q. Write short answer on contents of bone marrow.

BOX 10.9: Contents of bone marrow

- Hematopoietic cells
 - Myeloid series
 - Erythroid series
 - Megakaryocytes
 - Other cells: Lymphocytes, plasma cells

Contd...

Contd...

- Fat tissue
- Vessels
- Nerves
- Reticuloendothelial cells
- Stroma

Erythropoietin (EPO)

Q. Write short answer on erythropoietin.

It is a glycosylated protein synthesized mainly by kidney and minor part from liver. It is produced in response to hypoxia. EPO acts on the erythroid precursors through EPO receptors. This stimulates proerythroblast to proliferate and differentiate to produce RBCs.

Parasites Causing Anemia

Q. Write short note on parasites causing anemia.

- Parasites causing anemia
 - *Plasmodium: falciparum, vivax, ovale, malariae*
 - *Diphyllobothrium latum*
 - *Leishmania donovani*
 - *Ancylostoma duodenale*
 - *Necator americanus*
 - *Taenia solium*

Disorders of White Cells

QUANTITATIVE AND QUALITATIVE DISORDERS OF LEUKOCYTES

NORMAL DIFFERENTIAL LEUKOCYTE COUNT

Differential leukocyte count (DLC) is one of the routine, useful and important investigations.

The normal range of DLC in an adult is presented in Table 11.1.

TABLE 11.1: Normal range of different leukocytes in an adult

Type of white blood cell	Normal range
Neutrophils	40–70% ($2.0-7.0 \times 10^9/L$)
Lymphocytes	20–40% ($1.0-3.0 \times 10^9/L$)
Monocytes	2–10% ($0.2-1.0 \times 10^9/L$)
Eosinophils	1–6% ($0.02-0.5 \times 10^9/L$)
Basophils	Less than 1% ($0.02-0.1 \times 10^9/L$)

QUANTITATIVE DISORDERS OF LEUKOCYTES

Leukocytosis

Q. Define leukocytosis and list its causes.

An increase in the **total** number of **leukocytes** in the blood **more than 11,000/cu mm** ($11 \times 10^9/L$).

Leukocytosis is usually due to increase in the neutrophils, but may also be due to increased lymphocytes (or rarely monocytes and eosinophils).

Causes: Common causes of leukocytosis are shown in Box 11.1.

BOX 11.1: Common causes of leukocytosis

- Infections
 - Bacterial
 - Viral infections (e.g. infectious mononucleosis)
- Leukemia
 - Acute
 - Chronic: Chronic lymphocytic leukemia and chronic myeloid leukemia
- Leukemoid reactions
- Physiological
 - Pregnancy
 - Exercise

Leukopenia

Q. Write short note on leukopenia.

Total leukocyte count is less than 4,000/cu mm ($4 \times 10^9/L$).

Causes: Common causes of leukopenia are shown in Box 11.2.

Leukopenia is the decrease in the WBC count below 4,000/cu mm.

The causes of leukopenia include typhoid and paratyphoid fever and aplastic anemia.

BOX 11.2: Common causes of leukopenia

- Typhoid and paratyphoid
- Anemia
 - Aplastic anemia
 - Megaloblastic anemia
- Hypersplenism
- Drugs including cytotoxic drugs
- Radiation
- Rarely leukemia

Disorders of Neutrophils

Q. Define neutrophilia and mention its causes.

Neutrophilia (Fig. 11.1)

An absolute neutrophil count of more than 8000/cu mm ($8 \times 10^9/L$). Differential count shows more than 70% neutrophils and is usually accompanied by leukocytosis ($15\text{--}30 \times 10^9/L$).

Causes of Neutrophilia (Box 11.3).

Neutrophilia: Absolute neutrophil count more than 8000 cells/mm.

Common causes of neutrophilia are infections, inflammatory conditions and tissue necrosis.

Neutrophils in bacterial infections show toxic granules.

Dohle bodies are small round to oval structures seen in the cytoplasm, can also be observed in bacterial infections.

Leukemoid Reaction

Benign leukocytic proliferation characterized by a total leukocyte count of more than $50 \times 10^9/L$ with immature white cells (like band forms, metamyelocytes and myelocytes).

Leukemoid reaction: Benign exaggerated leukocyte proliferation to be differentiated from leukemia.

It is characterized by a reactive increase in the white blood cell and the blood picture closely resembles various types of leukemia.

Types of leukemoid reactions

1. **Myeloid leukemoid reaction:** Various causes include

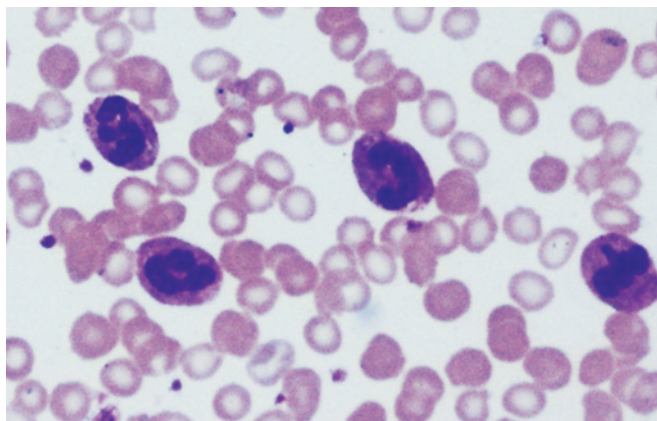


Fig. 11.1: Peripheral smear showing neutrophilia

BOX 11.3: Major causes of neutrophilia

1. Pathological:

- Acute bacterial and fungal infections:
 - Localized: Pyogenic microorganisms causing infections, e.g. pneumonias, pyogenic meningitis, cellulitis, diphtheria, abscess, tonsillitis, etc.
 - Generalized: Septicemia, acute rheumatic fever
- Acute inflammatory processes: Inflammatory conditions (acute appendicitis), vasculitis
- Tissue necrosis: Burns, myocardial infarction, gangrene, neoplasms (tumor necrosis)
- Acute stress or hypoxic states: Following hemorrhage, hemolysis and surgery
- Myeloproliferative neoplasms: Chronic myeloid leukemia, polycythemia vera
- Metabolic: Uremia, acidosis, gout
- Miscellaneous: Eclampsia, steroid therapy

2. Physiological:

- Exercise (shift from marginating pool to circulating pool), newborns, extremes of temperature, pain, emotional stress and during obstetric labor

- a. **Severe bacterial infections:** For example, staphylococcal pneumonia, endocarditis, meningitis, septicemia
- b. **Intoxications:** For example, eclampsia, severe burns, mercury poisoning
- c. **Malignancy:** For example, multiple myeloma, Hodgkin lymphoma, bone marrow metastasis
- d. **Severe hemorrhage and hemolysis.**

It should be differentiated from chronic myelocytic/myeloid leukemia (Table 11.2).

2. **Lymphoid leukemoid reaction:** Various causes include:

a. Infections:

- **Viral:** For example, infectious mononucleosis, cytomegaloviral infections
- **Bacterial:** Tuberculosis, whooping cough.

b. Malignancy.

Blood picture of lymphoid leukemoid reaction: Shows leukocytosis (not exceeding $100,000/\mu L$) and mostly mature lymphocytes resembling the blood picture of chronic lymphocytic leukemia.

Neutropenia (Agranulocytosis)

Q. Write short note on agranulocytosis.

Neutropenia: Absolute neutrophil count below 1500 cells/cu mm.

Reduction in the absolute neutrophil count (total WBC \times % segmented neutrophils and band forms) below $1.5 \times 10^9/L$ ($1500/\text{cu mm}$).

Q. Tabulate the differences between leukemia and leukemoid reaction.**TABLE 11.2:** Differences between leukemoid reaction and chronic myeloid leukemia

	<i>Leukemoid reaction</i>	<i>Chronic myeloid leukemia</i>
Clinical features	Features of causative disease	Splenomegaly, and bone pain are common
Peripheral blood findings		
WBC		
Total WBC count	Moderately increased, rarely exceeds $50 \times 10^9/L$	Markedly increased and usually $50 \times 10^9/L$
Differential leukocyte count	Shift to the left with few immature forms. Toxic granulation seen	Shift to the left with numerous immature forms. Myelocyte and neutrophil peak
Eosinophilia and basophilia	Variable	Present
Leukocyte alkaline phosphatase (LAP)	Increased	Decreased
RBC		
Anemia	Usually minimal or absent	Severe and progressive
Platelets		
Number	Variable	Normal or increased
Extramedullary myeloid tumors	Absent	Present
Philadelphia chromosome	Absent	Present

In leukemoid reaction, LAP score is raised and neutrophils may show toxic granulation.

Etiology

Causes of neutropenia are described in Box 11.4.

Eosinophilia (Fig. 11.2)**Q. Write short essay/note on eosinophilia—definition and its causes.**

Eosinophilia: Eosinophil count more than 450 cells/cu mm.

Eosinophil count of more than 450/cu mm ($0.45 \times 10^9/L$).

Causes of eosinophilia are presented in Table 11.3.

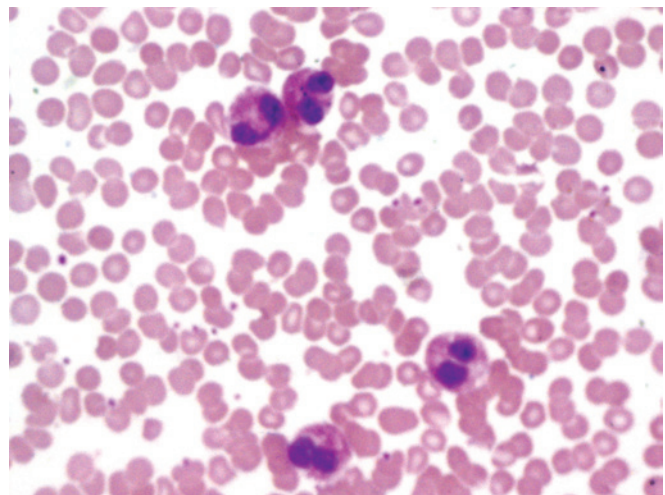
Basophilia**Q. Write short essay/note on basophilia and its causes.**

Normally basophils (Fig. 11.3) are less than 1% of WBCs in peripheral blood.

Causes of basophilia include chronic myeloid leukemia, immediate hypersensitivity reactions, mastocytosis, viral infections (e.g. smallpox, chickenpox) etc.

Monocytosis (Box 11.5)

More than 10% of differential count or an absolute monocyte (Fig. 11.4) count exceeding 500/cu mm ($0.5 \times 10^9/L$).

**Fig. 11.2:** Peripheral smear showing eosinophilia**Lymphocytosis****Q. Write short essay/note on lymphocytosis and its causes.**

Lymphocyte (Fig. 11.5) count more than 4,000/cu mm ($4 \times 10^9/L$) in adults and more than 8,000/cumm ($8 \times 10^9/L$) in child.

BOX 11.4: Causes of neutropenia**1. Inadequate production:**

- **Suppression of stem cells:** In these disorders granulocytopenia represents a component of pancytopenia
 - Aplastic anemia
 - Metastatic tumors
 - Marrow infiltration
 - Granulomatous disorders
- Suppression of committed granulocytic precursors
 - Drugs and chemicals (e.g. sulfonamides, analgesics, arsenicals)
 - Ionizing radiation
- Diseases associated with ineffective hematopoiesis
 - Megaloblastic anemias: Vitamin B₁₂ or folate deficiency
 - **Congenital:** Kostmann syndrome (rare)
 - **Myelodysplastic syndromes**
 - **Severe infections**
 - ◆ Bacterial (e.g. typhoid, paratyphoid, septicemia)
 - ◆ Viral (e.g. influenza, infectious mononucleosis, hepatitis, measles)
 - ◆ Rickettsial (e.g. scrub typhus)
 - ◆ Protozoal (e.g. malaria, kala-azar)

2. Increased destruction of neutrophils:

- Immunologically mediated destruction
 - Idiopathic
 - Secondary
 - ◆ Drugs
 - ◆ Autoimmune disorders, e.g. systemic lupus erythematosus
- **Splenic sequestration** may be associated with pancytopenia

3. Shift from the circulating pool to marginating pool:

- Hemodialysis and cardiopulmonary bypass

4. Idiopathic: mechanism not known

- Hodgkin and non-Hodgkin lymphoma
- Viral infections (HIV, hepatitis)
- Chronic lymphocytic leukemia
- Cyclic neutropenia

Agranulocytosis: Neutrophil count below $0.5 \times 10^9/L$. The patients are highly susceptible to bacterial and fungal infections.

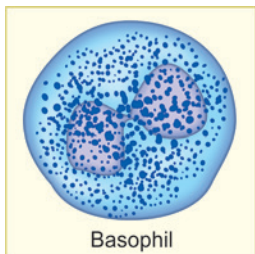


Fig. 11.3: Diagrammatic appearance of basophil

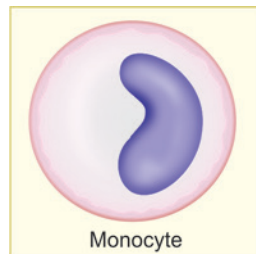


Fig. 11.4: Diagrammatic appearance of monocyte

Lymphocytosis: Lymphocyte count more than 4,000/cu mm in adults and more than 8,000/cu mm ($8 \times 10^9/L$) in child.

Common causes of lymphocytosis (Box 11.6).

Lymphocytopenia

Lymphocyte count below 1,500/cu mm ($1.5 \times 10^9/L$) in adults and below 3000/cu mm ($3 \times 10^9/L$) in children.

Some of the important causes of lymphocytopenia are listed in the Box 11.7.

Leukoerythroblastic Reaction/Blood Picture

Q. Write short note on leukoerythroblastic reaction/ blood picture and its causes.

It is the presence of immature white blood cells (myelocytes, metamyelocytes and promyelocytes) as well as nucleated RBCs in the peripheral blood.

Causes:

- **Infectious diseases:** Miliary tuberculosis
- **Bone metastasis:** For example, carcinoma of lung, breast, prostate, GI tract
- **Hematological conditions:** Myelofibrosis, severe hemolysis (e.g. erythroblastosis fetalis), multiple myeloma, lymphoma
- **Storage disorders:** Gaucher's disease, Niemann-Pick disease.

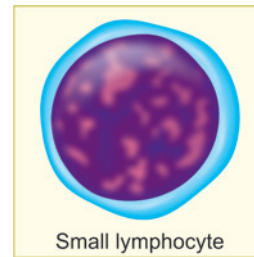
TABLE 11.3: Causes of eosinophilia

1. Allergic/atopic conditions	
• Asthma	• Urticaria
• Hay fever	• Drug reactions
• Allergic rhinitis	
2. Parasitic infestations (with tissue invasion)	
• Roundworm infestation	• Hookworm infestation
• Filariasis	
3. Fungal infections (e.g. coccidioidomycosis)	
4. Skin diseases	
• Dermatitis (eczema)	• Pemphigus
• Scabies	• Dermatitis herpetiformis
5. Hematological diseases	
• Chronic myeloid leukemia	• Polycythemia
• Hodgkin lymphoma	• Acute myelomonocytic leukemia
• Eosinophilic leukemia	
6. Miscellaneous	
• Tropical eosinophilia	• Pulmonary eosinophilia
• Löffler's syndrome	• Hypereosinophilic syndrome
• Eosinophilic granuloma	

Eosinophilia is seen in allergic reactions and parasitic infestations with tissue invasion.

BOX 11.5: Causes of monocytosis

- Infections**
 - Bacterial: Tuberculosis, bacterial endocarditis, brucellosis
 - Protozoal: Malaria, kala-azar
 - Spirochetal: Syphilis
 - Rickettsial: Typhus, rocky mountain fever
 - Recovery phase of neutropenia and acute infections
- Inflammatory diseases**
 - Inflammatory bowel disease: Ulcerative colitis, Crohn disease
 - Autoimmune diseases: Systemic lupus erythematosus, rheumatoid arthritis
 - Sarcoidosis
- Hematologic malignancies**
 - Acute monocytic, myelomonocytic and myelocytic leukemias
 - Chronic myelomonocytic leukemia
 - Hodgkin lymphoma
 - Multiple myeloma

**Fig. 11.5:** Diagrammatic appearance of lymphocyte**BOX 11.6:** Causes of lymphocytosis

- Acute infections**
 - Viral infections: Infectious mononucleosis, mumps, measles, chickenpox, infectious hepatitis
 - Toxoplasmosis
- Chronic infections/inflammatory diseases**
 - Tuberculosis
 - Syphilis
 - Brucellosis
 - Inflammatory bowel disease: Crohn disease and ulcerative colitis
- Hematologic malignancies**
 - Acute lymphoblastic leukemia
 - Chronic lymphocytic leukemia
 - Non-Hodgkin lymphoma with spill over
 - Adult T-cell leukemia/lymphoma
 - Hairy cell leukemia

Dengue fever is caused by flavi virus transmitted by freshwater mosquito (*Aedes aegypti*). Peripheral smear shows transformed lymphocytes and thrombocytopenia.

BOX 11.7: Causes of lymphocytopenia

- Increased destruction**
 - Corticosteroids
 - Cytotoxic drugs
 - Radiation
- Decreased production**
 - Aplastic anemia
 - Advanced malignancy: Hodgkin lymphoma
 - Infections: AIDS, miliary tuberculosis
- Increased loss via GI tract**
 - Obstruction to intestinal lymphatic drainage (e.g. tumor)
 - Congestive heart failure

QUALITATIVE DISORDERS OF LEUKOCYTES

Q. Write short note on qualitative/functional disorders of leukocytes.

Qualitative disorders of leukocytes are rare familial disorders that manifest as morphologic changes in the leukocytes (Fig. 11.6).

INFECTIOUS MONONUCLEOSIS (GLANDULAR FEVER)

Q. Write short essay/note on infectious mononucleosis.

Acute, benign, self-limiting lymphoproliferative disorder caused by Epstein-Barr virus (EBV).

- **Incubation period:** 4–8 weeks.
- **Mode of transmission:** Oropharyngeal secretions (kissing), hence the nickname kissing disease.

Q. Write short note on qualitative disorders of leukocytes.



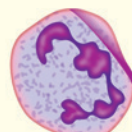
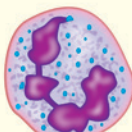

Pathogenesis

- EBV infects B lymphocytes by binding to CD21 (CR2) receptor.
- Viral infection begins in the submucosal lymphoid tissues of nasopharynx and oropharynx.
- Virus remains dormant inside the B-cells.
- B-cells are “immortalized” and are capable of proliferation indefinitely.

EBV infects B-cells but the peripheral blood shows CD8 + T-cells, which appear as atypical lymphocytes.

Clinical Features

- **Age:** Young adults among upper socioeconomic classes in developed nations and children of low socioeconomic status.
- **Signs and symptoms:** Classical triad
 - Fever

Qualitative abnormality	Mode of inheritance	Characteristic morphology	Functional abnormalities	Appearance of leukocyte
Pelger-Huet anomaly	Autosomal dominant	Lack of neutrophil nuclear segmentation beyond 2 lobes	Normal	
Alder-Reilly anomaly	Autosomal recessive	Large lilac inclusions in cytoplasm of all leukocytes	Normal	
May-Hegglin anomaly	Autosomal dominant	Large basophilic inclusions in all leukocytes. Giant platelets and thrombocytopenia	Abnormal bleeding due to thrombocytopenia	
Chediak-Higashi anomaly	Autosomal recessive	Large gray blue granules in cytoplasm of monocytes and granulocytes. Defective lysosomal granules	Poor chemotaxis Increased susceptibility to pyogenic infections Bleeding tendency	
Chronic granulomatous disease of childhood (CGD)	X-linked autosomal recessive	Normal appearance but defective function	Deficient NADPH oxidase, with absent H ₂ O ₂ production Phagocytosis and killing of organisms is impaired	

Chediak-Higashi anomaly is associated with increased susceptibility to pyogenic infections.

CGD is associated with impaired phagocytosis and killing of organisms.

Fig. 11.6: Various quantitative disorders of leukocytes

- Pharyngitis (sore throat)
- Lymphadenopathy.

Lesions caused by EBV:

- Infectious mononucleosis
- Burkitt lymphoma
- Nasopharyngeal carcinoma
- Hodgkin lymphoma
- X-linked lymphoproliferative disorders, and
- Body cavity lymphoma.

Laboratory Finding

Q. Mention the laboratory findings in infectious mononucleosis.

- **Total leukocytes count increased** (12,000–25,000 cells/cu mm): **Absolute lymphocytosis.**
- **Atypical lymphocytosis (mononuclear cells):** These are CD8 + subset (cytotoxic) of T-cells and not the virus-infected B-cells.
- **Serological tests**
 - **Demonstration of heterophile antibodies**
 - ◆ Paul Bunnell test is characteristically positive.
 - ◆ Monospot test is a sensitive slide test.
 - **Demonstration specific antibodies against EBV antigens:**
 - ◆ Antibody against viral capsid antigens (anti-VCA).
 - ◆ Antibodies to Epstein-Barr nuclear antigen (EBNA).

Demonstration of specific antibodies to EBV is the most specific test for infectious mononucleosis.

Normally blast cells are less than 5% of nucleated cells in the marrow.

Leukemia: Malignant disease of bone marrow stem cell, arises in the marrow and spreads.

Acute leukemia are mainly divided into two groups, namely acute lymphoblastic leukemia (ALL) and acute myeloblastic leukemia (AML).

Etiology and Pathogenesis

Q. Write short essay/note on etiology of leukemias.

Risk Factors (Box 11.8) may cause mutations in the proto-oncogenes and tumor suppressor genes.

BOX 11.8: Risk factors for acute leukemia

ENVIRONMENTAL FACTORS

- **Ionizing radiation**
- **Drugs:**
 - Alkylating agents—nitrogen mustard, chlorambucil, etc.
 - AML occurs in myeloma patients treated with melphalan
 - Leukemia follows chemotherapy of lung and ovarian cancer
- **Chemicals:** Benzene (used in paint industry, plastic glues, etc.)

GENETIC DISORDERS

Example: Down syndrome (ALL or AML), Fanconi anemia (AML), ataxia telangiectasia (ALL, NHL)

ACQUIRED DISORDERS

- PNH and aplastic anemia may transform into acute leukemia
- AML may develop de novo or secondary to myelodysplastic syndrome (MDS)

ACUTE LEUKEMIA

DEFINITION

Q. Define leukemia?

Acute leukemia is a malignant disease of the bone marrow stem cell and its characteristic features are:

- **Bone marrow:** Diffuse replacement with proliferating neoplastic blast cells that fail to mature. **Blast cells more than 20%** (WHO criteria) of the nucleated cells in the marrow.
- **Peripheral blood:** Abnormal numbers and forms of immature white blood cells.

Aleukemic/subleukemic leukemia is characterized by very few/no blasts in the peripheral blood.

Classification

Q. Classify acute leukemia.

Traditional classification depending on microscopic appearance of the involved cell and the course of leukemias is presented in Box 11.9.

BOX 11.9: Traditional classification of leukemia

- **Acute leukemia**
 - Acute myelogenous/myeloblastic/myelocytic/myeloid leukemia (AML)
 - Acute lymphoblastic/lymphocytic leukemia (ALL)
- **Chronic leukemia**
 - Chronic myeloid leukemia (CML)
 - Chronic lymphocytic leukemia (CLL)

FAB Classification of Acute Leukemias

FAB criteria for the diagnosis of acute leukemia: Bone marrow should show a blast count of 30% or more.

- **First French, American and British (FAB) classification** (1976) was based on the (1) morphological and (2) cytochemical characteristics of blast cells.
- **Revised FAB classification** (Box 11.10). It includes:
 1. Morphology and cytochemistry of blast cells
 2. Immunophenotyping
 3. Cytogenetics
 4. Molecular genetics.

WHO Classification (2016) of Acute Leukemia (Box 11.11)

WHO classification of AML: Based on clinical, morphological, immunophenotypic and genetic features.

Minimum blast cells in bone marrow should be more than 20%.

Differences between Myeloblast and Lymphoblast (Table 11.14)

Q. List the differences between myeloblast and lymphoblast.

It is important to differentiate between lymphoblast and myeloblast because of difference in treatment and prognosis of AML and ALL.

BOX 11.10: Revised French, American and British (FAB) classification of acute leukemias

Acute Lymphoid Leukemia

L ₁	Small homogenous cells with inconspicuous nucleoli
L ₂	Large cells with variable size and 1–2 nucleoli
L ₃	Large, homogeneous cells with finely stippled chromatin and prominent nucleoli. Cytoplasm is basophilic and vacuolated

Acute Myeloid Leukemia

M ₀	Minimally differentiated AML
M ₁	AML without maturation
M ₂	AML with maturation
M ₃	Promyelocytic leukemia
M ₄	Myelomonocytic leukemia
M ₅	Monocytic leukemia
M ₆	Erythroleukemia
M ₇	Megakaryocytic leukemia

Cytochemistry in Leukemia

Q. Write short essay/note on cytochemistry in acute leukemia

Cytochemistry is the study of chemical elements found in the cytoplasm of the cells. The chemical element in the cell

BOX 11.11: WHO classification (2016) of acute lymphoid and myeloid leukemia

A. Acute lymphoid leukemia

I. B lymphoblastic leukemia/lymphoma

- B lymphoblastic leukemia/lymphoma, NOS
- B lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities
 - B lymphoblastic leukemia/lymphoma with t(9;22) (q34; q11.2); *BCR-ABL1*
 - B lymphoblastic leukemia/lymphoma with t(v;11q23.3); *KMT2A* rearranged
 - B lymphoblastic leukemia/lymphoma with t(12;21) (p13.2;q22.1); *ETV6–RUNX1*
 - B lymphoblastic leukemia/lymphoma with hyperdiploidy
 - B lymphoblastic leukemia/lymphoma with hypodiploidy
 - B lymphoblastic leukemia/lymphoma with t(5;14) (q31;q32); *IL3-IGH*
 - B lymphoblastic leukemia/lymphoma with t(1;19) (q23;p13.3); *TCF3-PBX1*

II. T lymphoblastic leukemia/lymphoma

B. Acute Myeloid Leukemia

I. AML with recurrent genetic abnormalities

- AML with t(8;21)(q22;q22); *RUNX1–RUNX1T1*
- AML with inv(16)(p13;q22); *CBFB–MYH11*
- APL with t(15;17)(q22;q12); *PML–RARA*
- AML with t(9;11)(p22;q23); *MLLT3–MLL*
- AML with t(6;9)(p23;q34); *DEK–NUP214*
- AML with inv(3)(q21q26.2) *RPN1–EVI1*
- AML(megakaryoblastic) with t(1;22) (p13;q13); *RBM15–MKL1*
- AML with mutated *NPM1*
- AML with mutated *CEBPA*

II. AML with MDS-related changes

III. Therapy-related myeloid neoplasms

IV. AML not otherwise specified

- AML minimally differentiated (M1)
- AML without maturation (M2)
- AML with maturation (M3)
- Acute myelomonocytic leukemia (M4)
- Acute monoblastic and monocytic leukemia (M5)
- Acute erythroid leukemia (M6)
- Acute megakaryoblastic leukemia (M7)

V. Myeloid sarcoma

VI. Myeloid proliferation related to Down syndrome

Abbreviations: AML, Acute myeloid leukemia; APL, Acute promyelocytic leukemia; MDS, Myelodysplastic syndrome

TABLE 11.4: Differences between myeloblast and lymphoblast based on morphology and cytochemistry

	<i>Lymphoblast (Figs 11.7, 11.12 and 11.13)</i>	<i>Myeloblast (Figs 11.8, 11.14 and 11.15)</i>
Size	2–3 times the size of lymphocyte	3–5 times the size of lymphocyte
Cytoplasmic characters		
Amount	Scanty (less cytoplasm than myeloblast)	Scanty to moderate (more cytoplasm than lymphoblast)
Color	Blue	Gray
Cytoplasmic granules	Agranular	May have cytoplasmic granules
Auer rod	Negative	Positive
Nuclear characters		
Nuclear chromatin	Uniform, coarse	Uniform, fine
Nucleoli	Inconspicuous or 1–2	3–5, prominent
N:C ratio	High	High
Accompanying cells	Lymphocytes	Promyelocytes, myelocytes, meta-myelocytes, band forms and neutrophils
Cytochemistry		
Myeloperoxidase	Negative	Positive
Sudan Black	Negative	Positive
PAS	Block positivity	Negative
Nonspecific esterase	Negative	Positive in M4 and M5

Myeloblast: Comparison with lymphoblast has 4 Ms

M: More in size

M: More nucleoli (3–5)

M: Moderate cytoplasm

M: Myeloperoxidase positive

Auer rod: positive

may be an enzyme (e.g. myeloperoxidase) or non-enzymatic substance (e.g. lipids and glycogen).

Uses

- To characterize the blast cells as myeloid or lymphoid in acute leukemias. Sometimes, differentiating AML from ALL is not possible only on morphological grounds and in such cases cytochemical stains will be of help.
- To identify granulocytic and monocytic components of acute myeloid leukemia.
- To detect cytoplasmic abnormalities and enzyme deficiencies in myeloid disorders.

Myeloperoxidase

- Myeloperoxidase (MPO) is an enzyme present in the primary granules of **mature and immature** cells of **myeloid series** (Fig. 11.10).
- **Principle:** When hydrogen peroxide is present, the myeloperoxidase within the cytoplasm oxidizes the dye substrate creating brown granules in the cytoplasm and the intensity of positivity increases with maturity of the cell.
- **Interpretation:** (1) Leukemic myeloblasts are positive (AML M1, M2, M3 and M4 blasts and Auer rods). (2) Lymphoblasts and normoblasts are negative. Thus, useful in differentiating AML from ALL.

Sudan Black B

- **Principle:** Sudan black B (SBB) stains lipids and phospholipids present in the granules of the cells of **myeloid series** (Fig. 11.11). The granules take up black color. Staining pattern in MPO and SBB are similar.

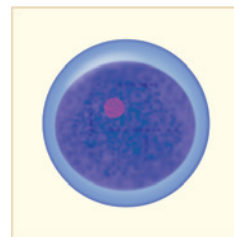


Fig. 11.7: Diagrammatic appearance of lymphoblast

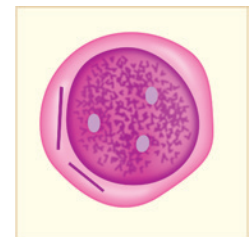


Fig. 11.8: Diagrammatic appearance of myeloblast



Fig. 11.9: Periodic acid Schiff (PAS) stain showing lymphoblast with block positivity

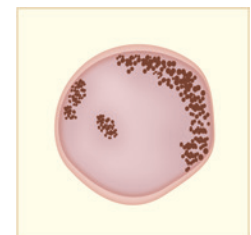


Fig. 11.10: Myeloblast stained positively with myeloperoxidase (MOP)

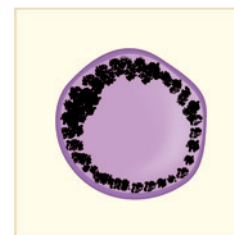


Fig. 11.11: Myeloblast stained positively with Sudan Black

- **Interpretation:** Useful in differentiation of AML from ALL.

Nonspecific Esterase

- Esterase is used to differentiate **myeloblast, neutrophilic precursors and neutrophilic granulocytes** from monocytic cells.
- **Principle:** Esterases are enzymes in cells of monocytic series and are used to differentiate them from myeloblasts and lymphoblasts.
- **Interpretation:** Esterase is useful for identification and confirmation of the monocytic component in AML M4 and M5. Monocytic cells show dark red cytoplasmic granules.

Periodic Acid-Schiff Reaction

- Periodic acid-Schiff (PAS) stain is useful for the diagnosis of ALLs, and erythroid and megakaryocytic (M6 and M7) type of acute myeloid leukemia.
- **Principle:** Many cells contain glycogen in their cytoplasm. The periodic acid oxidizes glycogen, mucoproteins and other high molecular weight (HMW) carbohydrates to aldehydes. These aldehydes react with colorless Schiff reagent and take bright-red pink (*magenta*) color. The staining pattern may be fine and diffuse, coarse and granular (block positivity), or a mixture of both patterns.
- **Interpretation:** Useful for differentiating AML from ALL.
 - Myeloblasts are negative. Erythroid type of acute myeloid leukemia may show PAS—positive erythroblasts.
 - **Lymphoblasts show blocks of PAS positive** (Figs 11.9 and 11.12 inset) material in the cytoplasm. Block positivity in lymphoblasts is observed in most of the ALL L1 cases.

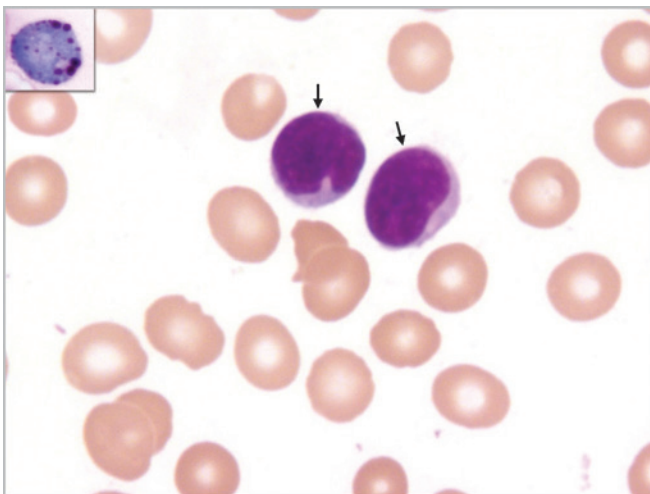


Fig. 11.12: Peripheral blood smears in acute lymphoblastic leukemia showing lymphoblasts (arrows). Inset shows lymphoblast with block positivity with PAS stain

Neutrophil Alkaline Phosphatase

- **Principle:** Alkaline phosphatase is an enzyme located in the specific (secondary) or tertiary granules present in the cytoplasm of mature neutrophils, with some activity in metamyelocytes. The positive reaction gives rise to blue and granular appearance to the cytoplasmic granules.
- Normal NAP/LAP score: 40–100.
- **LAP increased** in (1) hematological disorders such as leukemoid reaction, myelofibrosis, polycythemia rubra vera, idiopathic thrombocythemia and (2) non-hematological disorders such as pregnancy and Down syndrome
- **LAP decreased** in paroxysmal nocturnal hemoglobinuria and chronic myeloid leukemia

ACUTE LYMPHOBLASTIC LEUKEMIA/ LYMPHOMA

Differentiating malignant pre-B and pre-T lymphoblasts on morphology is difficult.

- **Acute Lymphoblastic Leukemia/Lymphoma (ALL)** is a group of neoplasms consisting of **lymphoblasts**.
- Lymphoblast is immature, precursor B (pre-B) or T (pre-T) lymphocyte.
- WHO classification (Box 11.11):
 - **B lymphoblastic leukemia/lymphoma** (about 85%) seen in **childhood** and present as **acute leukemias**.
 - **T lymphoblastic leukemia/lymphoma** (15%) present in adolescent males as **lymphomas**, often with involvement of mediastinum (thymus).

Molecular Pathogenesis

- Chromosomal abnormalities are found in about 90% of ALLs.
 - **Numerical abnormality:** Hyperploidy (>50 chromosomes) and hypoploidy.
 - **Structural abnormality:** Balanced chromosomal translocations (e.g. Philadelphia chromosome).
 - ♦ Most T-ALLs have mutations in NOTCH1 gene.
 - ♦ Most B-ALLs have mutations in genes PAX5, E2A and EBF or a balanced translocation t (12; 21) involving the genes TEL and AML1.

Requires immunophenotyping for subclassification of ALL.

T-ALL has worse prognosis compared to B-ALL.

Classification of Acute Lymphoblastic Leukemia (Box 11.10, 11.11 and Table 11.5)

Clinical Features

ALL is the most common leukemia in children and is usually associated with lymphadenopathy.

Age: Most common hematological malignancy of children. Most common between 1 and 5 years of age and between 30 and 40 years.

Sex: Slight male preponderance.

Onset: Abrupt.

Symptoms:

Symptoms are due to bone marrow infiltration by blasts.

Bone marrow failure:

(1) anemia, (2) neutropenia, and (3) thrombocytopenia.

- **Bone marrow failure:**
 - Anemia: causes **fatigue, weakness**.
 - Neutropenia: **Infections** by bacteria or opportunistic fungi. Develop sore throat and respiratory infections.
 - Thrombocytopenia: **Bleeding** into the skin and mucosa in the form of purpura or ecchymoses.
 - **Bone pain and sternal tenderness.**
- **Extramedullary infiltration:**
 - **Lymphadenopathy:** 75% of patients, usually involve cervical lymph nodes.
 - **Bone pain and tenderness.**

- **Hepatosplenomegaly:** Splenomegaly is more common than hepatomegaly.
- **Mediastinal thymic mass:** More common in T-ALL.
- **CNS involvement:** Spread into the meninges causes leukemic meningitis ALL (pre-B).
- Testicular involvement (ALL).

Laboratory Findings

Q. Write short note on laboratory/peripheral smear findings in acute lymphoblastic leukemia.

Peripheral Blood

- **Total WBC count:** markedly raised ranging from $20 \times 10^9/L$ to $200 \times 10^9/L$
- **Platelet count:** reduced (thrombocytopenia).
- **Hemoglobin:** decreased and may be as low as 3 g/dL.
- **Peripheral smear** (Figs 11.12 and 11.13):
 - **RBCs:** Normocytic normochromic anemia.
 - **WBCs:** Total count **markedly increased** and **20% or more lymphoblasts**.
 - ♦ **Morphology of lymphoblasts:**
 - ◇ Larger than small lymphocyte
 - ◇ High N:C ratio
 - ◇ Nucleus with condensed chromatin and nucleoli are either absent or inconspicuous
 - ◇ Scant to moderate agranular basophilic cytoplasm.
 - **Platelets:** Thrombocytopenia.

TABLE 11.5: Characteristics of FAB subtypes of acute lymphoid leukemias (ALL)

FAB type	L_1	L_2	L_3
Cell size	Small cell size	Large heterogeneous cell population	Large, homogeneous cell population
Nuclear characteristics			
Shape	Regular	Irregular, clefting and indentation common	Regular, oval or round
Chromatin	Condensed	Dispersed chromatin	Finely stippled
Nucleolus	Small and inconspicuous	Visible, 1–2 in number	Usually prominent
Cytoplasmic characteristics			
Amount	Scanty	Variable, often abundant	Moderately abundant
Cytoplasmic basophilia	Slight to moderate	Variable	Strong
Cytoplasmic vacuolation	Absent	Variable	Prominent and oil red O stain positive

ALL- L_1 has better prognosis than ALL- L_3 .

Morphologically, as per the FAB classification lymphoblast are classified as L_1 , L_2 and L_3 .

ALL- L_3 is a leukemic counterpart of Burkitt lymphoma.

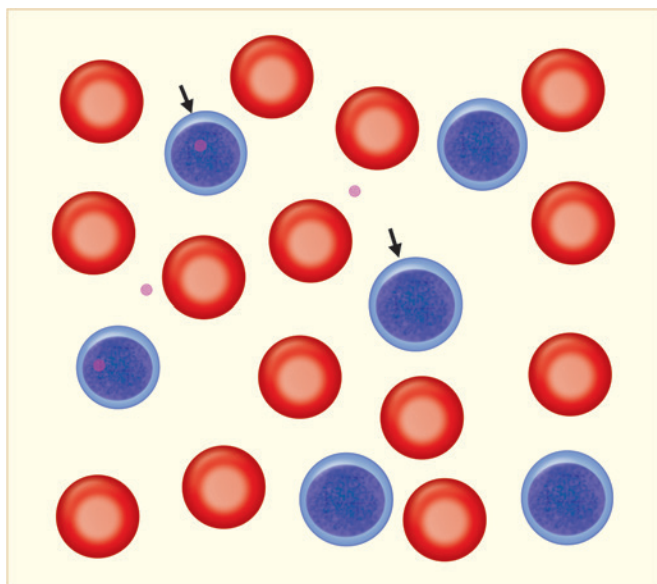


Fig. 11.13: Diagrammatic peripheral blood smear in acute lymphoblastic leukemia showing lymphoblasts (arrows)

Subleukemic leukemia: Total WBC count lower than $4 \times 10^9/L$ and peripheral blood shows very few blasts.

Aleukemic leukemia: Total white cell count is low ($< 4 \times 10^9/L$) with no blasts in the peripheral blood.

Lymphoblasts should be differentiated from myeloblasts (see Table 11.14).

Cytochemistry of Lymphoblasts

Lymphoblast: Cytoplasm shows block positivity with PAS stain.

- **PAS:** Cytoplasmic aggregates of **PAS positive** (Figs 11.9 and 11.12) material (**block positivity**).
- **Myeloperoxidase (MPO)** negative.
- **Sudan black B** negative.

BONE MARROW

- **Cellularity:** Markedly **hypercellular** due to proliferation of blasts.
- **Erythropoiesis and myelopoiesis:** reduced.
- **Megakaryopoiesis:** Megakaryocytes gradually decrease.
- **Blasts:** constitute 20–100% of the marrow cells.

Immunophenotyping

Terminal-deoxynucleotidyl-transferase (TdT) + in pre-B and pre-T lymphoblasts.

Distinction between precursor B- and T-cell ALL requires lineage-specific markers.

TABLE 11.6: Prognostic factors in ALL

	Unfavorable prognosis	Favorable prognosis
Age	Below 2 years and above 10 years (adolescence or adulthood)	Between 2 to 10 years
Sex	Males	Females
Total WBC count	High (more than 50,000 cells/cu mm)	Low
Blast count in peripheral blood	Greater than 100,000 cells/cu mm	Lesser than 100,000 cells /cu mm
Meningeal involvement	Present	Absent
Cytogenetic abnormalities	t(9;22) (the Philadelphia chromosome)	Hyperploidy, trisomy of chromosomes 4, 7 and 10 and t(12;21)
Time required for clearing blasts from blood	More than 1 week	Less than 1 week

Prognosis is far better in ALL than AML.

- Immature B-cells + positive for pan B cell marker CD19 and CD10 (CALLA—common ALL antigen).
- Precursor T ALL cells are positive for CD2, CD5 and CD8.

Biochemical Findings

- **Serum uric acid:** Raised due to destruction of leukemic cells during chemotherapy leading to hyperuricemia.
- **LDH:** Raised, because of increased turnover of leukemic cells.

CSF Examination

To know/rule out CNS involvement.

Prognosis: Prognostic features of ALL are presented in Table 11.6.

Presence of Philadelphia chromosome in ALL: Prognosis unfavorable.

ACUTE MYELOGENOUS LEUKEMIA

Definition: Neoplasm of hematopoietic progenitors characterized by proliferation resulting in **accumulation of immature myeloblasts in the marrow**.

Classification of acute myelogenous leukemia (AML): Refer Box 11.10 and 11.11.

AML synonyms: Acute myeloid/myeloblastic/myelocytic leukemia.

Molecular Pathogenesis

- Many recurrent genetic abnormalities can disrupt genes encoding transcription factors involved in normal myeloid differentiation.
- **Mutated tyrosine kinase activation** is a common.

Clinical Features

Age: AML may develop at **any age**, but is more **common in adults**.

AML: develop at any age. Usually 15–60 years of age.

Onset: Acute leukemias are **abrupt in onset**.

Symptoms: Related to depressed marrow function.

- **Bone marrow failure:**
 - Anemia: Fatigue and weakness.
 - Neutropenia: Life-threatening infections by bacteria or opportunistic fungi.
 - Thrombocytopenia: Bleeding, patient may also develop disseminated intravascular coagulation (DIC) in AML M3 and primary fibrinolysis.
 - Bone pain and tenderness.
- **Extramedullary infiltration**
 - Gingival hypertrophy (M4 and M5) and infiltration of skin (leukemia cutis).
 - Hepatosplenomegaly: Usually more than in ALL.

Symptoms are due to anemia, neutropenia and thrombocytopenia.

Acute promyelocytic leukemia (AML-M3) may be associated with widespread bleeding due to DIC.

Laboratory Findings

Q. Write short note on laboratory/peripheral smear findings in AML.

Peripheral Blood

- **Total WBC Count:** Markedly raised, ranging from $20 \times 10^9/L$ to $100 \times 10^9/L$.
- **Hemoglobin:** Decreased and ranges from 5 to 9 g/dL.

Q. Write short note on Auer rods.

- **Peripheral smear** (Figs 11.14 and 11.15):
 - **RBCs:** Normocytic normochromic type of anemia.
 - **WBCs:** Total WBC **count markedly increased**.
 - ♦ Differential count: **More than 20% myeloid blasts**. May show more than one type of blast or blasts with hybrid features.
 - ♦ **Morphology of myeloblasts:**
 - ◊ 3 to 5 times larger than the diameter of a small lymphocyte.
 - ◊ High N:C ratio.
 - ◊ Fine nuclear chromatin with 2–4 variably prominent nucleoli.
 - ◊ More cytoplasm than lymphoblasts—azurophilic, peroxidase-positive granules.
 - ◊ Presence of Auer rods is definitive evidence of myeloid differentiation.
 - ♦ **Auer rods** are azurophilic needle-like peroxidase-positive structures in the cytosol of myeloblasts (M_2 and M_3 subtype).
 - **Platelets:** Moderate to severe thrombocytopenia and causes bleeding from skin and mucosa.

AML: Auer rods in the cytoplasm of myeloblasts, seen in AML; not in CML.

Cytochemistry of Myeloblasts

(Figs 11.10 and 11.11)

Q. Write short note on special stains in AML.

- Stain **positively** with **myeloperoxidase (MPO)** and **Sudan black B**.
- Monoblasts stain with nonspecific esterases.

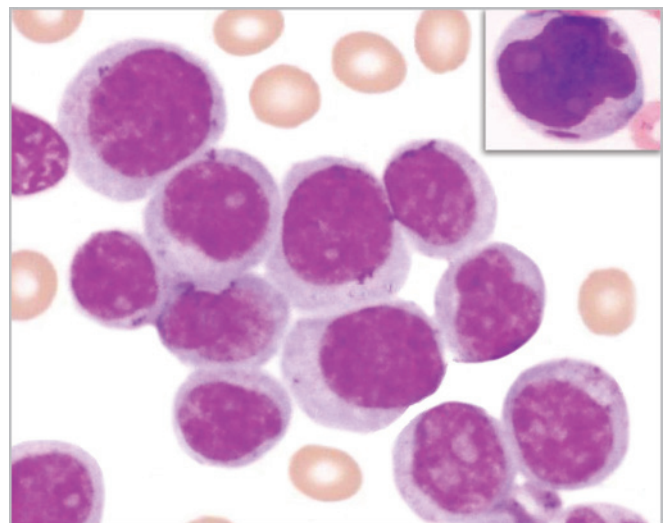


Fig. 11.14: Peripheral smear in AML with myeloblasts. Inset shows myeloblast with Auer rod

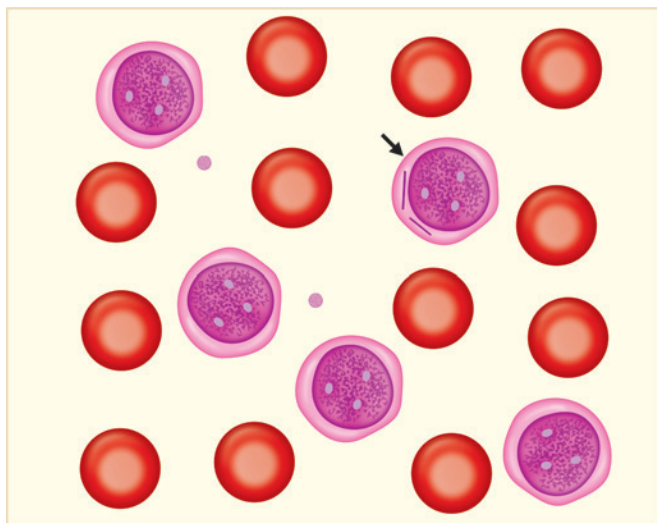


Fig. 11.15: Diagrammatic peripheral blood smear in AML with myeloblasts. One myeloblast with two Auer rods (arrow)

Myeloblasts stain positively with myeloperoxidase and Sudan black B.

BONE MARROW

- **Cellularity:** Markedly hypercellular.
- **Erythropoiesis:** Markedly suppressed.
- **Myelopoiesis:** Suppression of myeloid maturation and myeloblasts constitute more than 20% of marrow cells.
- **Megakaryopoiesis:** Gradually decreased.

Both in subleukemic and aleukemic leukemia bone marrow contains blasts more than 20%.

Immunophenotyping

Diagnosis of AML is confirmed by using stains for myeloid specific antigens.

AML prognosis:

- Fulminant course and has worse prognosis than ALL.
- Cytogenetic markers are major determinants of prognosis.

Cytogenetics

Very important in the WHO classification of AML (See Box 11.11).

Myeloid sarcoma synonym: Extramedullary myeloid tumor/granulocytic sarcoma/chloroma.

MYELOID SARCOMA

Tumor mass consisting of myeloid blasts with or without maturation occurring at extramedullary sites.

- **On sectioning:** Tumor is green (hence the term chloroma)
- **Microscopically myeloblasts** with or without features of promyelocytic or neutrophilic maturation.

Myeloid sarcoma is frequent in skin, lymph node, GI tract, bone, soft tissue and testis.

MYELOYDYSPLASTIC SYNDROMES

INTRODUCTION

Q. Write short essay/note on myelodysplastic syndrome.

Myelodysplastic Syndromes (MDS) are a heterogeneous group of **acquired clonal stem cell disorders affecting stem cells**.

MDS: Cytopenias with hypercellular bone marrow.

About 30% progress to AML.

MDS is characterized by:

- **Progressive cytopenias**
- **Dysplasia** in one or more cell lines
- **Ineffective hematopoiesis**
- **Risk of development of AML.**

Classification

- **Idiopathic or primary MDS**
- **Secondary/therapy-related MDS (t-MDS):** Complication of previous cytotoxic drug or radiation therapy.

WHO classification of myelodysplastic syndromes is presented in Box 11.12.

Clinical Features

- Elderly above 60 years
- Slightly more common in males
- Symptoms are due to cytopenias
- About 10%–40% of MDS patients progress to AML.

BOX 11.12: WHO (2016) classification of myelodysplastic syndromes

- MDS with single lineage dysplasia
- MDS with ring sideroblasts
 - MDS with ring sideroblasts and single lineage dysplasia
 - MDS with ring sideroblasts and multilineage dysplasia
- MDS with multilineage dysplasia
- MDS with excess blasts
- MDS with isolated del(5q)
- MDS, unclassifiable

Laboratory Findings

- **Peripheral smear:** Cytopenias in the peripheral blood
 - *RBCs*: Mild to moderate degree of macrocytic or dimorphic anemia.
 - *WBCs*: Normal or low total leukocyte count.
 - *Platelets*: Variable thrombocytopenia, large hypogranular or giant platelets.

BONE MARROW

Dysplasia of all nonlymphoid lineages (erythroid, granulocytic, monocytic and megakaryocytic) associated with cytopenias.

- **Cellularity:** Hypercellular.
 - **Erythropoiesis:** Dysplastic changes in erythroid precursors with **megaloblastoid** change and presence of **ringed sideroblasts** in iron stain.
 - **Myelopoiesis:** Hyperplasia with dysgranulopoiesis.
 - **Megakaryopoiesis:** Dysmegakaryopoiesis—paw ball megakaryocytes.
 - **Iron stores:** Increased with ring sideroblasts.
- Ineffective hematopoiesis

Bone marrow in MDS: Paw ball megakaryocytes, dysgranulopoiesis, erythroid precursors with megaloblastoid change and presence of ringed sideroblasts.

Bone Marrow Trephine Biopsy

Abnormal localization of immature precursors (ALIP) in (refractory anemia with excess blasts (RAEB).

MYELOPROLIFERATIVE NEOPLASMS

INTRODUCTION

Q. Write short note on myeloproliferative neoplasms.

Definition: Clonal hematopoietic stem cell disorders characterized by **proliferation of one or more of the myeloid lineages** (erythroid, granulocytic, megakaryocytic and mast cells).

- **Splenomegaly** and **hepatomegaly** due to sequestration of excess hematopoietic cells or proliferation of abnormal hematopoietic cells.

All MPN show splenomegaly.

MPN peaks in the 5th to 7th decade.

WHO Classification of MPN

It is presented in Box 11.13.

BOX 11.13: WHO (2016) classification of myeloproliferative neoplasm (MPN)

WHO (2008) Myeloproliferative neoplasms

Chronic myelogenous leukemia, BCR-ABL-1 positive

Chronic neutrophilic leukemia

Polycythemia vera—JAK2 V617F or exon 12 mutation

Primary myelofibrosis—JAK2 or MPL mutation

Essential thrombocythemia

- Platelet count $> 450 \times 10^9/L$
- JAK2 mutation

Chronic eosinophilic leukemia, NOS

- No BCR-ABL1, PDGFRA, PDGFRB or FGFR1 translocation

Myeloproliferative neoplasm, unclassifiable

*Mastocytosis is no longer listed under the broad heading of MPN in WHO (2016) classification.

Pathogenesis

Presence of mutated, constitutively activated tyrosine kinases leads to proliferation of hematopoietic stem cells and results in hypercellular marrow.

POLYCYTHEMIA OR ERYTHROCYTOSIS

Polycythemia is characterized by **increase in the RBC mass**, usually with a corresponding increase in hemoglobin level.

Pathophysiologic classification of polycythemia is given in Box 11.14.

BOX 11.14: Pathophysiologic classification of polycythemia

ABSOLUTE

Primary (low erythropoietin level)

- Polycythemia vera

Secondary (high erythropoietin level)

- Physiologically appropriate
 - Compensatory
 - Lung disease
 - Living in high-altitude
 - Cyanotic heart disease (Tetralogy of Fallot)
- Physiologically inappropriate (with increased erythropoietin)
 - Paraneoplastic: erythropoietin-secreting tumors (e.g. renal cell carcinoma, uterine leiomyoma, hepatocellular carcinoma)

RELATIVE

Reduced plasma volume

- Hemoconcentration (dehydration due to diarrhea, vomiting)
- Gaisböck's syndrome (spurious polycythemia)

Polycythemia: Increase in red cells can be absolute or relative.

POLYCYTHEMIA VERA

Q. Write short note on polycythemia vera.

Definition: Polycythemia vera (PV) is an **acquired myeloproliferative neoplasm** arising from malignant transformation of **hematopoietic stem cell**.

- It is characterized by **trilineage** (erythroid, granulocytic, and megakaryocytic) **hyperplasia** in the bone marrow.
- It leads to **uncontrolled production of red cells, granulocytes and platelets** (panmyelosis) and leads to **erythrocytosis** (polycythemia) and or granulocytosis and thrombocytosis.

Molecular Pathogenesis (Figs 11.16 and 11.17)

- Normally, a tyrosine kinase protein called JAK2 (Janus 2 kinase gene), is activated following binding of the growth hormone erythropoietin.
- JAK2 then activates a signaling pathway causing cells to replicate.
- This process is strictly regulated by various feedback pathways.
- Polycythemia vera (PV) is due to **mutation in** tyrosine kinase **JAK2 V617F**, which causes proliferation of not only erythroid lineage but also granulocytic and megakaryocytic lineage.

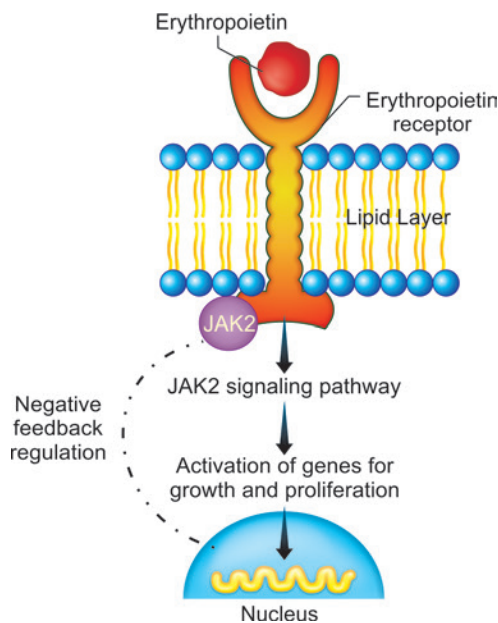


Fig. 11.16: Normal signaling by JAK2

JAK2 mutation is diagnostic of polycythemia vera.

PV: Erythropoietin is decreased.

Clinical Features

PV: Most symptoms are due to the increased red cell mass and hematocrit.

- Insidious.
- Late middle age (median age at onset is 60 years).
- Plethora and cyanosis, headache, dizziness and visual problems result from vascular disturbances in the brain and retina.
- Thrombotic episodes: For example, deep venous thrombosis, myocardial infarction, thrombosis of hepatic veins (producing Budd-Chiari syndrome).

Phases

There are three phases of polycythemia vera:

1. **Proliferative phase:** Erythroid proliferation and increased red cell mass.
2. **Spent phase:** In 10%, excessive proliferation of erythroid cells ceases with stable or decreased RBC mass.
3. **Myelofibrosis:** About 10% progress to myelofibrosis.

PV develops into acute myelogenous leukemia in 2%–5%.

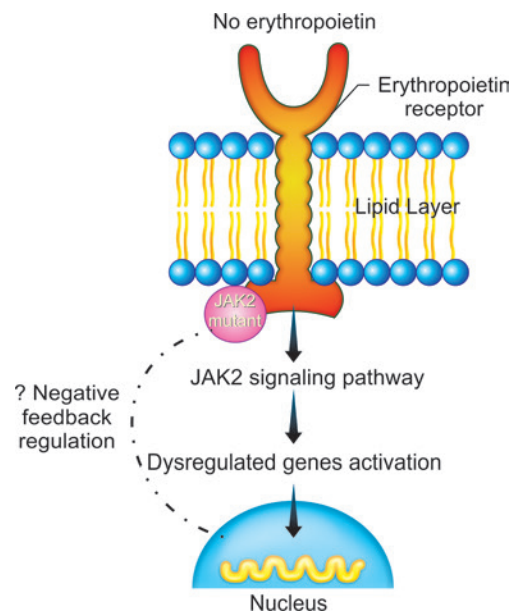


Fig. 11.17: In polycythemia vera, the presence of a mutant version of JAK2 results in dysregulated downstream signaling in the absence of erythropoietin

Laboratory Findings

Peripheral Blood (Fig. 11.18)

- **Hemoglobin:** Increased and are more than 18.5 g/dL in men and 16.5 g/dL in women.
- **Hematocrit:** Increased and about 60%.
- **Red cell count:** Increased and usually about 6 million/cu mm ($6 \times 10^{12}/L$).
- **White cell count:** Normal or increased.
- **Platelet count:** Normal or increased.

Polycythemia vera is a chronic myeloproliferative neoplasm with RBC count of more than 6 million/cu mm.

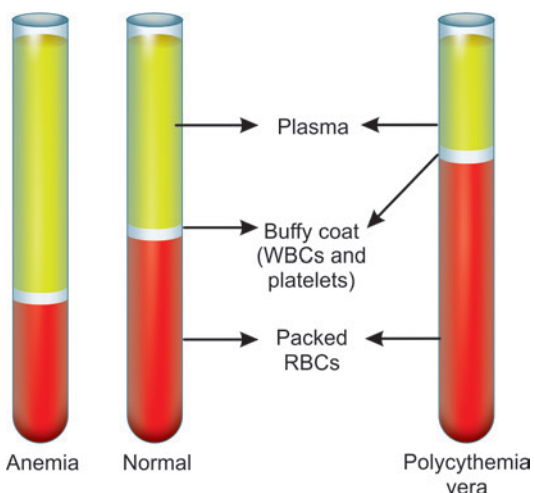
- **Peripheral smear:**
 - **RBCs:** Normocytic normochromic picture.
 - **WBCs:**
 - ◆ **Mild to moderate leukocytosis**
 - ◆ Neutrophils are morphologically normal
 - ◆ **Basophils often increased**
 - ◆ **NAP (LAP) score is increased** to 150–300 (Normal 40–100).
 - **Platelets:** **Abnormally large and functionally defective.**

BONE MARROW

- Hypercellular due to **hyperplasia of all elements** (trilineage hyperplasia/panmyelosis) namely erythroid, myeloid and megakaryocytic series with **prominence of erythroid precursors** in the bone marrow.

Bone Marrow Biopsy

Shows increased reticulin fibers and fibrosis as the disease progresses.



PV: Hematocrit is increased and >60%.

Fig. 11.18: Normal hematocrit in comparison with anemia and polycythemia vera

Other Findings

In PV, arterial oxygen saturation (pO_2) is normal (>92%) whereas in secondary polycythemia, it is <90%.

- **Extramedullary hematopoiesis** in the liver and spleen that causes **hepatosplenomegaly**.
- **Arterial oxygen saturation (pO_2):** Normal (75–100 mm Hg) and is useful for **differentiating it from secondary polycythemia**.
- **Erythropoietin levels:** **Decreased** in contrast to secondary polycythemia.
- **Serum vitamin B₁₂ and uric acid:** **Increased** indicating increased cell turnover.
- **JAK2 V617F mutation:** Can be demonstrated.

ESSENTIAL THROMBOCYTHEMIA

Essential thrombocythemia (ET) synonym: Primary (essential/idiopathic) thrombocytosis.

Definition: **Chronic myeloproliferative neoplasm (MPN)** primarily of **megakaryocytic lineage**. It is characterized by **increased megakaryopoiesis** and **thrombocytosis** (more than $450 \times 10^9/L$).

Etiology

ET: Mutation of JAK2 gene

Thrombocytosis with a count of $>450 \times 10^9/L$.

- Most due to point **mutations in JAK2 gene** and constitutive activation of JAK2, and thrombopoietin-independent proliferation of megakaryocytes.

Clinical Features

ET: Throbbing and burning sensation of hands and feet due to blocking of arterioles by aggregates of platelets is known as **erythromelalgia**.

- Age: 50–60 years
- **Thrombosis and hemorrhage**
- **Erythromelalgia:** One of the characteristic features.

Laboratory Findings

- **Peripheral smear:**
 - **RBCs:** Normocytic normochromic.
 - **WBCs:** **Mild leukocytosis.**
 - **Platelets:**
 - ◆ **Increased number (thrombocytosis)** $> 600,000/cu\ mm$.
 - ◆ Variation in size and shape—abnormally large platelets are common.

Megakaryocytic hyperplasia and abnormal (giant) platelets are characteristic features.

BONE MARROW

- *Cellularity*: Mild to marked hypercellularity.
- *Erythropoiesis*: Normal or mild hyperplasia.
- *Myelopoiesis*: Normal or mild hyperplasia.
- *Megakaryopoiesis*: **Markedly increased** in number with **abnormally large megakaryocytes (giant megakaryocytes)**.

ET course: Indolent.

Extramedullary hematopoiesis: Mild hepatosplenomegaly.

PRIMARY MYELOFIBROSIS

Q. Write short essay/note on myelofibrosis.

Myelofibrosis: Mutation in JAK2 gene.

Clonal MPN characterized by a **proliferation of predominant megakaryocytes and granulocytes** in the bone marrow.

Fully developed disease results in **reactive marrow fibrosis** and replaces hematopoietic cells leading to cytopenias and extensive extramedullary hematopoiesis.

Molecular Pathogenesis

Most show JAK2 mutations.

Clinical Features

Massive splenomegaly due to extramedullary hemopoiesis.

- **Age**: Above 60 years of age.
- Progressive anemia.
- Splenomegaly.

Laboratory Findings

Primary myelofibrosis: Peripheral smear shows leukoerythroblastosis and tear drop cells.

- **Peripheral smears**:
 - *RBCs*: Moderate to severe degree of **normochromic normocytic anemia** accompanied by **leukoerythroblastosis**. **Teardrop-shaped red cells** (dacryocytes), probably due to damage in the fibrotic marrow can also be found.
 - *WBCs*: Total white cell count is usually normal or reduced, but can be markedly elevated $80\text{--}100 \times 10^9/\text{L}$ in early stages of the disease.
 - *Platelets*: They may be **abnormally large**. The **platelet count is usually normal** or elevated, but as the disease progresses the count decreases.

Primary myelofibrosis: Bone marrow fibrosis leads to cytopenias.

BONE MARROW

- *Cellularity*: In early stages, it is often hypercellular due to increase in maturing cells of all lineages. In later stages, it is replaced by fibrosis and becomes hypocellular and diffusely fibrotic resulting in a **dry tap**.
- *Erythroid and granulocytic precursors*: These are morphologically **normal**.
- *Megakaryocytes*: These are **large, dysplastic and abnormally clustered**.

Bone Marrow Biopsy

Bone marrow biopsy is essential for the diagnosis of myelofibrosis as aspirate results in a dry tap late in the course of the disease.

Stages: Two stages have been recognized.

1. **Prefibrotic (cellular) stage**: Hypercellular bone marrow. Megakaryocytes increased and markedly abnormal.
2. **Fibrotic stage**: Fibrosis distorts the marrow and prematurely releases nucleated erythroid and early granulocyte progenitors (**leukoerythroblastosis**).

Reticulin stain demonstrates the increase in reticulin fibers (fibrosis).

Extramedullary hematopoiesis in spleen and liver **produces hepatosplenomegaly**.

Course: Variable.

CHRONIC MYELOGENOUS LEUKEMIA

CML synonyms: Chronic myelocytic/myeloid/granulocytic leukemia.

Q. Discuss peripheral blood smear, bone marrow, biochemical findings and the characteristic chromosomal abnormality associated with chronic myeloid leukemia.

Definition

CML is an acquired MPN of pluripotent hematopoietic stem cell.

Chronic myelogenous leukemia (CML) is one of the **myeloproliferative neoplasm (MPN)** of **pluripotent hematopoietic stem cell** characterized by overproduction of cells of the myeloid series which results in marked splenomegaly and leukocytosis.

Distinguished from other myeloproliferative neoplasms by the presence of:

1. Chimeric fusion BCR-ABL1 gene.

- Philadelphia (Ph) chromosome in more than 90% of cases.

Etiology and Pathogenesis

Risk factor: Exposure to ionizing radiation and benzene.

Molecular Pathogenesis

Q. Write short note on Philadelphia chromosome.

Philadelphia (Ph) Chromosome (Fig. 11.19)

- Acquired chromosomal abnormality in **all proliferating hematopoietic stem cells** (erythroid, myeloid, monocytic and megakaryocytic precursors).
- Balanced reciprocal translocation** between long arm of chromosome 9 and 22, i.e. **t (9; 22) (q 34; q 11.2)**. It increases the length of chromosome 9 and shortening of 22. This **shortened chromosome 22** is known as Philadelphia chromosome (Fig. 11.19).

Philadelphia (Ph) chromosome is a shortened chromosome 22 and is due to balanced reciprocal translocation between chromosome 9 and 22-t (9; 22).

BCR-ABL1 Fusion Gene (Fig. 11.20)

- ABL1** proto-oncogene from chromosome 9 **joins** the **BCR** on chromosome 22.

Translocation results in a BCR-ABL1 fusion gene, which produces neoplastic proliferation.

- It produces a new **chimeric (fusion) gene** called **BCR-ABL1**, thus **converting ABL1 proto-oncogene into oncogene**. The product of the fusion gene plays a central role in the development of CML.
- The product of this oncogene, i.e., **oncoprotein** (e.g. **p210**) causes cell division and inhibition of apoptosis.

Clinical Features

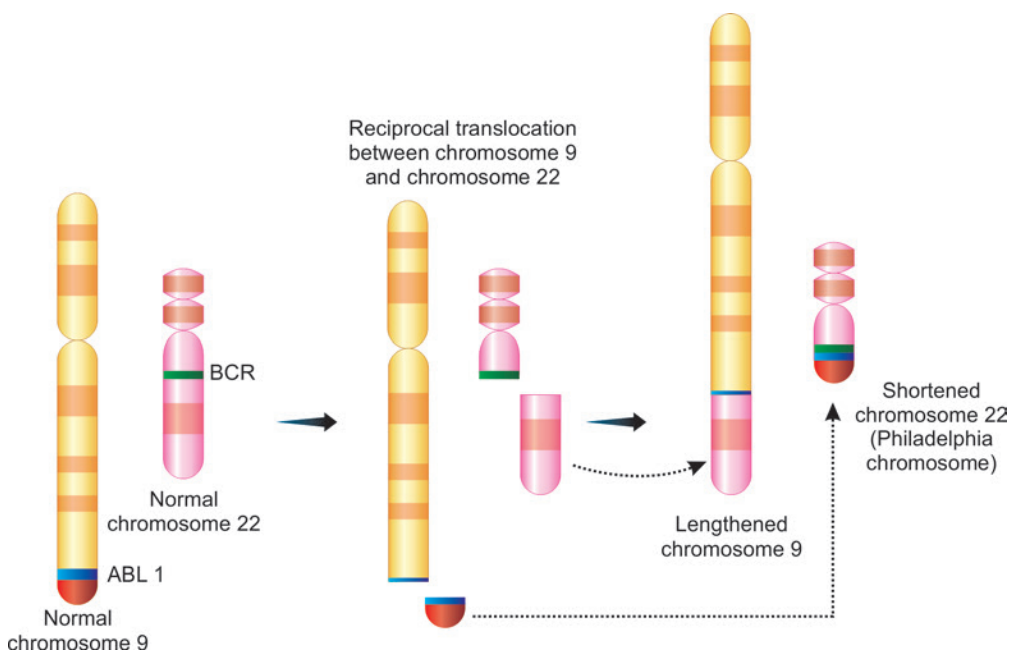
CML: Usually occurs between 40 and 60 years of age.

- Age:** Usually occurs between 40 and 60 years of age.
- Sex:** Males slightly more affected than females.
- Onset:** Insidious.

Symptoms:

- Nonspecific symptoms:** Fatigue, weakness, weight loss, anorexia.
- Fullness of abdomen due to splenomegaly** (caused by leukemic infiltration and extramedullary hematopoiesis). Splenomegaly is moderate to severe and is characteristic feature in majority (80–90%) of patients.
- Hepatomegaly:** Mild or moderate seen in 60–70% of cases.

CML: Moderate to massive splenomegaly.



CML: Translocation results in the head-to-tail fusion of the breakpoint cluster region (BCR) gene on chromosome 22 with the ABL (named after the abelson murine leukemia virus) gene located on chromosome 9.

Fig. 11.19: Balanced reciprocal translocation between long arm of chromosome 9 and chromosome 22 resulting in shortened chromosome 22 known as Philadelphia chromosome

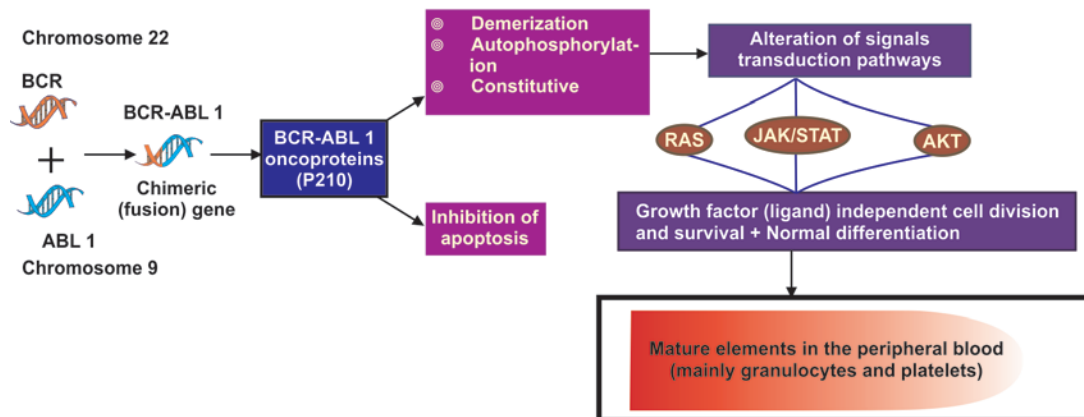


Fig. 11.20: Fusion of ABL1 gene from chromosome 9 with BCR on chromosome 22 and its consequences

Natural History of Chronic Myeloid Leukemia

CML has three phases: Chronic stable, accelerated and blast phase.

Three different phases: (1) chronic phase, (2) accelerated phase and (3) blastic phase.

Chronic/Stable/Indolent Phase (CP)

- **Most are diagnosed in this phase.**
- Lasts for 2–6 years.
- If not treated, progresses gradually to accelerated phase or abruptly to blastic phase.

Laboratory Findings

Q. Write short note on laboratory findings/peripheral smear in chronic myeloid leukemia (CML).

Peripheral blood

CML: Neutrophilia with the whole spectrum of mature myeloid precursors.

- **Hemoglobin:** Usually less than 11 g/dL
- **Peripheral smear:**
 - **RBCs:** Normocytic normochromic anemia
 - **WBCs:**
 - ♦ **Marked leukocytosis** ($12\text{--}600 \times 10^9/\text{L}$) total leukocyte count usually exceeds $100 \times 10^9/\text{L}$ (1,00,000/cu mm).

- ♦ **Shift to left (shift to immaturity)**—granulocytes at all stages of development (neutrophils, metamyelocytes, myelocytes, promyelocytes and an occasional myeloblasts).
- ♦ **Predominant cells are neutrophils and myelocytes.**
- ♦ **Blasts are usually less than 10%** of the circulating WBCs (Figs 11.21 and 11.22).
- ♦ **Basophilia and eosinophilia.**
- ♦ **Decreased NAP/LAP score:** NAP score in CML is decreased below 20 (normal score range is 40–100). Helpful in differentiating CML from leukemoid reaction (see Table 11.2).
- **Platelets:** Platelets range from normal ($150\text{--}450 \times 10^9/\text{L}$) to greater than $1000 \times 10^9/\text{L}$. Up to 50% have **thrombocytosis**.

CML is characterized by anemia, extreme leukocytosis, granulocytic immaturity, basophilia, thrombocytosis.

The preponderance of myelocyte is called as myelocyte bulge.

In CML, LAP (NAP) is markedly reduced.

BONE MARROW

- **Cellularity:** Markedly **hypercellular** due to **myeloid hyperplasia**.
- **M:E ratio:** Often exceeds **20:1**.
- **Erythropoiesis:** **Diminished erythropoiesis** as disease progresses.
- **Myelopoiesis:** **Marked hyperplasia**. Blast cells usually **less than 10%**. Basophils, eosinophils and their precursors are usually found.
- **Megakaryopoiesis:** Megakaryocytes are either normal or increased. **Dwarf megakaryocytes**.
- **Sea-blue histiocytes** (Gaucher-like cells/pseudo Gaucher cells) are seen.

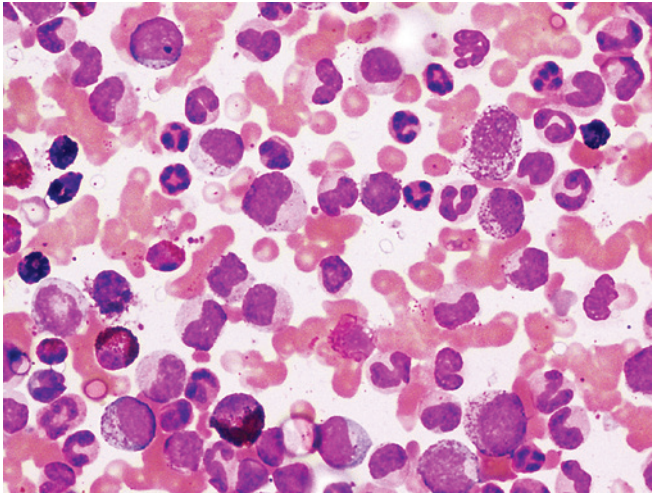


Fig. 11.21: Peripheral blood picture in chronic/stable phase of chronic myeloid leukemia

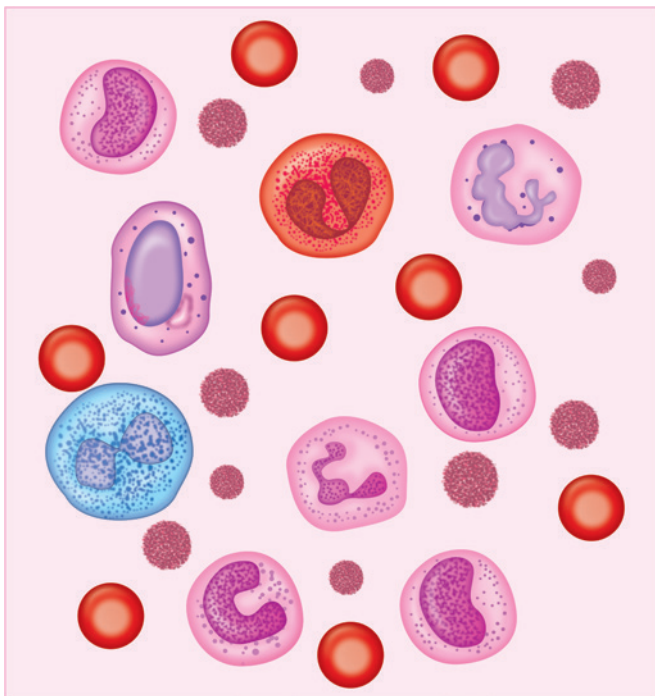


Fig. 11.22: Diagrammatic peripheral blood picture in chronic/stable phase of chronic myeloid leukemia

Biochemical Findings

- Serum uric acid raised
- Serum LDH raised.

Philadelphia chromosome and **BCR-ABL1 fusion gene** demonstrated either by chromosomal analysis or fluorescent in situ hybridization (FISH) or PCR based tests.

Accelerated Phase (AP)

CML: Accelerated phase is more aggressive and myeloblasts range from 10% to 19%.

- **More aggressive** and lasts for few months.
- **Myeloblasts: 10–19%** in the blood or bone marrow.
- **Striking basophilia** (20% or more).
- **Persistent thrombocytopenia** (less than $100 \times 10^9/L$) unrelated to therapy or persistent thrombocytosis (more than $1000 \times 10^9/L$) uncontrolled by therapy.
- **Megakaryocyte proliferation in sheets** or clusters in association with fibrosis.
- **Persistent or increasing splenomegaly** unresponsive to therapy.

Blast Phase/Crisis (BP)

CML blast crisis: Blasts 20% or more, myeloblast (no Auer rods) or lymphoblasts.

Blood picture resembles acute leukemia and has poor prognosis.

- **Peripheral smear (Figs 11.7 and 11.8):**
 - **Blasts 20% or more.** May be either myeloblast (70% cases) or lymphoblast (30% cases). Myeloblast does not contain Auer rods.
 - **Thrombocytopenia** causes bleeding episodes.

Prognosis: Poor with accelerated phase or blast crisis.

CHRONIC LYMPHOCYTIC LEUKEMIA/ SMALL LYMPHOCYTIC LYMPHOMA

CHRONIC LYMPHOCYTIC LEUKEMIA

Definition

Q. Write short note on chronic lymphocytic leukemia.

Chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL) is a **tumor composed of monomorphic small B lymphocytes** in the peripheral blood, bone marrow and lymphoid organs (spleen and lymph nodes).

- Both CLL and SLL is a **single entity with different presentations**.
- **Small lymphocytic lymphoma (SLL)** is tissue equivalent of chronic lymphocytic leukemia (CLL).
- CLL/SLL tumor cells coexpress CD5 and CD23.

CLL/SLL are tumors derived from B lymphocytes.

Etiology and Pathogenesis

- **Environmental factors:** Suggested but none proved.
- **Hereditary factors:** Families with higher risk of CLL or other lymphoid neoplasms.

Cytogenetic Abnormalities

Common mutations are deletions of 13q14.3, 11q22-23, and 17p11. About 20% of CLL show trisomy 12.

CLL patients may be asymptomatic or present with generalized lymphadenopathy.

Clinical Features

- **Age:** Between 50–60 years of age.
- **Sex:** More in males than in females (2:1).
- **Symptoms:**
 - **Asymptomatic** in about 25–30%
 - **Nonspecific symptoms:** Fatigue, loss of weight and anorexia
 - **Generalized lymphadenopathy**
 - **Immunological defects** either as immune deficiency or autoimmunity.

Laboratory Findings

Peripheral Blood

Q. Write short essay/note on peripheral blood picture in chronic lymphocytic leukemia.

CLL: Absolute lymphocyte count is more than $5 \times 10^9/L$. It is the characteristic feature.

- **Hemoglobin:** Decreased and usually below 13 g/dL.
- **Total leukocyte count is increased** ($20\text{--}50 \times 10^9/L$).
- **Peripheral smear** (Figs 11.23 and 11.24):
 - **RBCs:** Normocytic normochromic anemia.
 - **WBCs:**
 - ◆ Differential leukocyte count shows **lymphocytosis** and constitutes **more than 50%** of the white cells.
 - ◆ **Lymphocytes mature type**—small with scant cytoplasm, **nuclei round with clumped coarse chromatin** ("soccer ball"/**block-type chromatin**). Nucleoli absent.
 - ◆ **Smudge cells** or basket cells (fragile leukemic cells).
 - **Platelets:** Initially **normal** count and later may be decreased.

CLL: Lymphocytosis with smudge cells in the peripheral smear. Smudge cells are fragile leukemic cells produced due to rupture while making the peripheral smear.

Lymphocytes constitute more than 30% of the nucleated cells of the bone marrow cells—diagnostic feature of CLL.

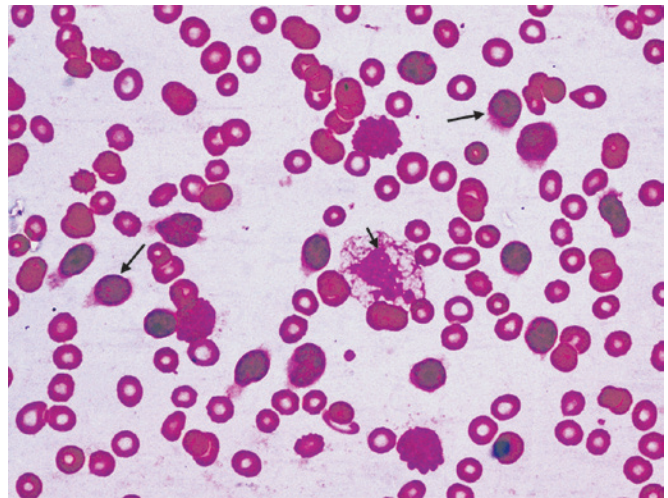


Fig. 11.23: Peripheral blood smear in chronic lymphocytic leukemia showing numerous small lymphocytes (long arrows) and few smudge cells (short arrow)

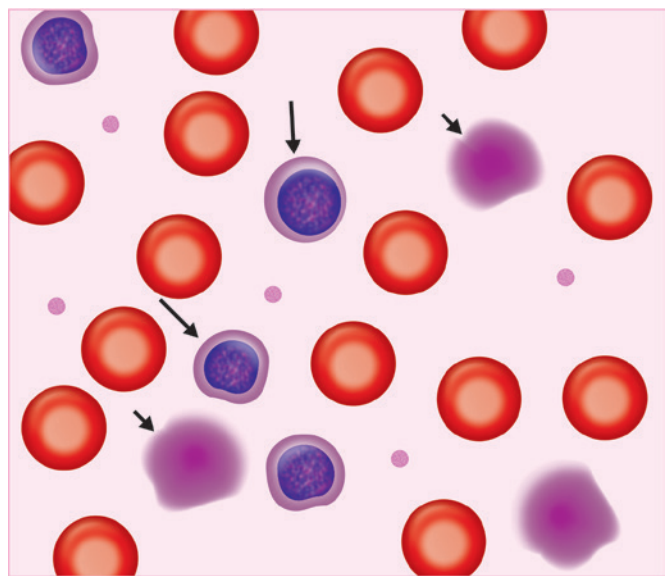


Fig. 11.24: Diagrammatic peripheral blood smear in chronic lymphocytic leukemia showing numerous small lymphocytes (long arrows) and few smudge cells (short arrows)

BONE MARROW

- **Cellularity:** **Hypercellular** marrow due to infiltration by mature lymphocytes.
- **Erythropoiesis:** Normal.
- **Myelopoiesis:** Normal.
- **Megakaryopoiesis:** Normal.
- **Lymphocytic infiltrate:** As the disease advances, neoplastic lymphocytes replace the normal erythroid, myeloid and megakaryocytic series in the bone marrow resulting in anemia, neutropenia and thrombocytopenia.

Immunophenotype

Tumor cells express the pan-B cell markers CD19 and CD20. CD5+ and CD23+ are distinctly positive in CLL.

Lymph Node

- Show loss of normal architecture.
- Diffuse infiltration by monomorphic, small, round lymphocytes.
- Lymphocytes have nuclei with coarse chromatin and scanty cytoplasm.
- Small, nodular aggregates of medium to large-sized lymphocytes known as **proliferation centers or pseudo-follicles or growth centers** and when found are **pathognomonic for CLL/SLL**.

CLL/SLL: Lymph node with proliferation centers are pathognomonic.

Monoclonal B-cell lymphocytosis (MBL): WHO criteria for monoclonal B-cell lymphocytosis (MBL) is the presence of monoclonal B-cell populations in the peripheral blood (PB) of up to $5 \times 10^9/L$ either with the phenotype of chronic lymphocytic leukemia (CLL), atypical CLL, or non-CLL (CD52) B-cells in the absence of other lymphomatous features. It has been found that MBL precedes almost all cases of CLL/SLL. In 2018 WHO subdivided MBL into “low count” MBL (characterized by PB CLL count of $<0.5 \times 10^9/L$) and “high count” MBL. The distinction is important because low count MBL does not require routine follow-up whereas, high count MBL requires routine/yearly follow-up.

Course and prognosis: Median survival rate is 4 to 6 years. They may progress to B-cell prolymphocytic transformation or into diffuse large B-cell lymphoma (Richter syndrome).

HAIRY CELL LEUKEMIA

Q. Write short essay/note on hairy cell leukemia.

Hairy cells have hair-like cytoplasmic projections.

Definition

Uncommon neoplasm of small mature B cells having abundant cytoplasm with fine **hair-like cytoplasmic projections** (hence the name **hairy cell leukemia**) when viewed under the **phase-contrast microscope**.

Laboratory Findings

HCL: Is B cell neoplasm and involves peripheral blood, bone marrow, spleen and liver and usually seen in old age.

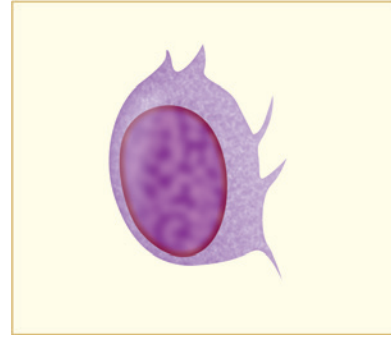


Fig. 11.25: Hairy cell

Peripheral Blood

- **Hemoglobin: Decreased.**
- **Total leukocyte count: Decreased (leukopenia).**
- **Platelet count: Decreased (less than $50 \times 10^9/L$)**

Peripheral smear: Pancytopenia

- RBCs: Normocytic normochromic.
- WBCs: Leukopenia with few **hairy cells** (Fig. 11.25).
- Platelets: Reduced.

BONE MARROW ASPIRATION

- **Dry tap**
- **Hairy cells** may be seen in the marrow
- Moderate to marked **reduction in myeloid, erythroid and megakaryocytic cell lines.**

Bone Marrow Trephine Biopsy

Neoplastic cells have “**fried egg**” or “**honeycomb**” appearance. Reticulin stain shows marked increase of thin reticulin fibers surrounding neoplastic cells.

HCL: Bone marrow biopsy—hairy cells have fried egg appearance.

Spleen

- **Enlarged** due to leukemic infiltrate.
- **Sinuses lined by hairy cells** and **grossly impart a beefy red appearance.**

Immunophenotype and Molecular Characteristics

Tartrate resistant acid phosphatase (TRAP) positivity in the cytoplasm is a characteristic feature of HCL.

Express the **CD20, CD22, CD11c and CD25** (the IL-2 receptor α -chain) **positivity**. Annexin A 1 is the most specific marker of hairy cell leukemia.

HCL: Only leukemia without lymphadenopathy.

Clinical Features

- Affects **middle-aged to elderly men**.
- Male-to-female ratio of 5:1.
- **Massive splenomegaly**.
- **Pancytopenia**.

HCL prognosis: Indolent course and prognosis is excellent.

PLASMA CELL NEOPLASMS

DEFINITION

Plasma cell neoplasms are group of B-cell neoplasms associated with the **proliferation of single clone** (monoclonal) of **immunoglobulin-secreting plasma cells** (also known as dyscrasias).

Characteristics of Plasma Cell Neoplasms

Plasma cell neoplasms: Tumor cells secrete single type of complete or fragment of immunoglobulins.

Q. Write short note on M proteins

Monoclonal neoplastic plasma cells **secrete complete single type of immunoglobulin (Ig) or Ig fragment**. Hence, are known as **monoclonal gammopathies**.

- **Serum:** Single Ig proteins detected as **monoclonal spike** [M protein (M for myeloma)] **on electrophoresis**.
- **Urine:** **Excess of free light chains** is excreted in the urine as **Bence-Jones (BJ) proteins**.

Classification of Plasma Cell Neoplasms (Box 11.15)

BOX 11.15: Classification of plasma cell neoplasms (WHO 2016)

- Plasma cell myeloma
- Solitary plasmacytoma of bone
- Extraosseous plasmacytoma
- Immunoglobulin deposition diseases
- Monoclonal gammopathy of undetermined significance (MGUS)
- Osteosclerotic myeloma (POEMS syndrome)

PLASMA CELL MYELOMA (MULTIPLE MYELOMA)

Definition

Multiple myeloma (MM) is a multifocal malignant tumor of plasma cell and arises in the bone marrow.

Plasma cell myeloma is a **malignant, multifocal plasma cell neoplasm of the bone marrow** associated with **M-protein** in the serum and/or urine.

- **Most common monoclonal gammopathy**.
- Presents as **multiple tumor masses** throughout the skeletal system.

Etiology

Plasma cell neoplasms arise from post-germinal center B-cells.

Risk Factors

- **Genetic predisposition**.
- **Exposure to ionizing radiation**.
- **Chronic antigenic stimulation** associated with chronic infections (HIV and chronic osteomyelitis) and chronic inflammatory disorders (e.g. rheumatoid arthritis).
- **Exposure to chemicals** like benzene, herbicides and insecticides.

Laboratory Findings

Q. Write short note on the laboratory diagnosis of multiple myeloma.

Peripheral Blood

- **Hemoglobin:** **Decreased** and ranges from 6 to 10 g/dL.
- **Peripheral smear:**
 - **RBCs:** Normocytic normochromic anemia, red blood cells show **rouleaux formation** due to increased immunoglobulins.
 - **WBCs:** **Normal**.
 - **Platelets:** **Normal**.
- **ESR:** **High** and is due to high gamma globulin (immunoglobulin) and rouleaux formation.
- **Bleeding time:** **Increased**.

MM: Hypergammaglobulinemia is responsible for high ESR and rouleaux formation seen in peripheral smear.

BONE MARROW

Q. Write short note on morphology of plasma cells in multiple myeloma.

Q. Write short note on bone marrow findings in multiple myeloma.

Bone marrow in MM: Hypercellular, and contains more than 30% neoplastic plasma cell.

- **Cellularity:** Hypercellular due to **myeloma plasma (myeloma) cells** (neoplastic plasma cells) (Figs 11.26 and 11.27).
- **Myeloma plasma cells:** More than 30% are diagnostic.
 - Myeloma plasma cells are neoplastic plasma cells (Fig. 11.26), which are **large oval cells** having **abundant pale blue cytoplasm**.
 - The **nucleus** is round to oval, **eccentric** and shows **peri-nuclear clearing/hof**.
 - The nuclear **chromatin** appears like a **clock-face/spoke wheel**.
 - These cells are usually uninucleated or may show binucleation.
 - Other cells can also be seen in myeloma (Fig. 11.28).

Myeloma plasma cells are commonly called as myeloma cells.

- **Erythropoiesis:** Diminished and is normoblastic.
- **Myelopoiesis:** Normal.
- **Megakaryopoiesis:** Normal.

Serum Findings

- **Serum β_2 microglobulin:** Prognostic marker and high values signify poor prognosis.
- **Hypercalcemia**
- **Renal function tests:** Blood urea, serum creatinine and uric acid levels are raised with renal involvement.
- **Serum albumin:** Decreases in advance stages of the disease.

Electrophoretic Studies on Serum and Urine (Figs 11.29 and 11.30)

Q. Write short note on urinary findings in multiple myeloma.

Q. Write short note on Bence-Jones protein and its demonstration.

- **Monoclonal spikes** in 80–90% of cases.
- Raised **monoclonal immunoglobulins** in the blood. Immunoglobulin may be **IgG** (most common)/IgD/IgA/IgE type.
- **Light chains** or **Bence Jones (BJ) proteins** in the urine may be seen in 60–80% of cases. BJ protein may be of κ or λ type of light chain.

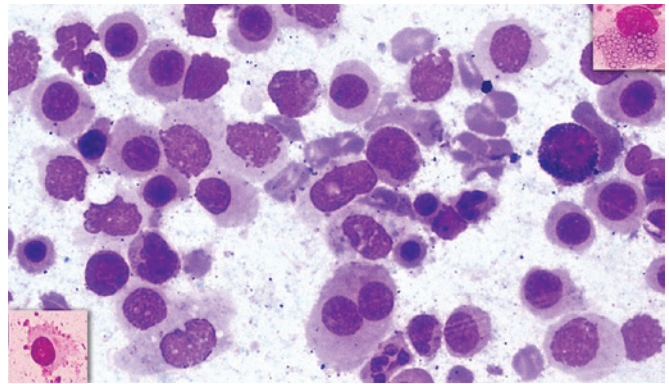


Fig. 11.26: Bone marrow aspirate in multiple myeloma, with numerous myeloma plasma cells. Inset shows flame cell (left lower corner) and mott cell (right upper corner)

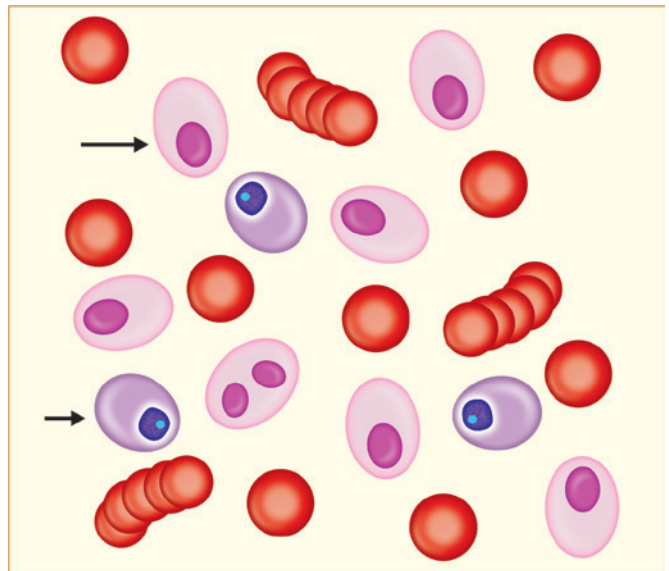


Fig. 11.27: Diagrammatic appearance of bone marrow in multiple myeloma showing plasmablasts (short arrow) and plasma cells (long arrow)

Morphology of Organs Involved

MM: IgG is the most common immunoglobulin secreted.

- **Bone:** Destructive **punched-out** lytic lesions (Fig. 11.31).
- **Renal lesions:**
 - **Myeloma kidney:** Light-chain cast of BJ protein damages renal tubules.
 - **Amyloidosis** of the AL type and leads to **nephrotic syndrome**.
 - ♦ Hypercalcemia leads to nephrocalcinosis.
 - ♦ Prone to acute and chronic pyelonephritis.
 - ♦ Renal failure.

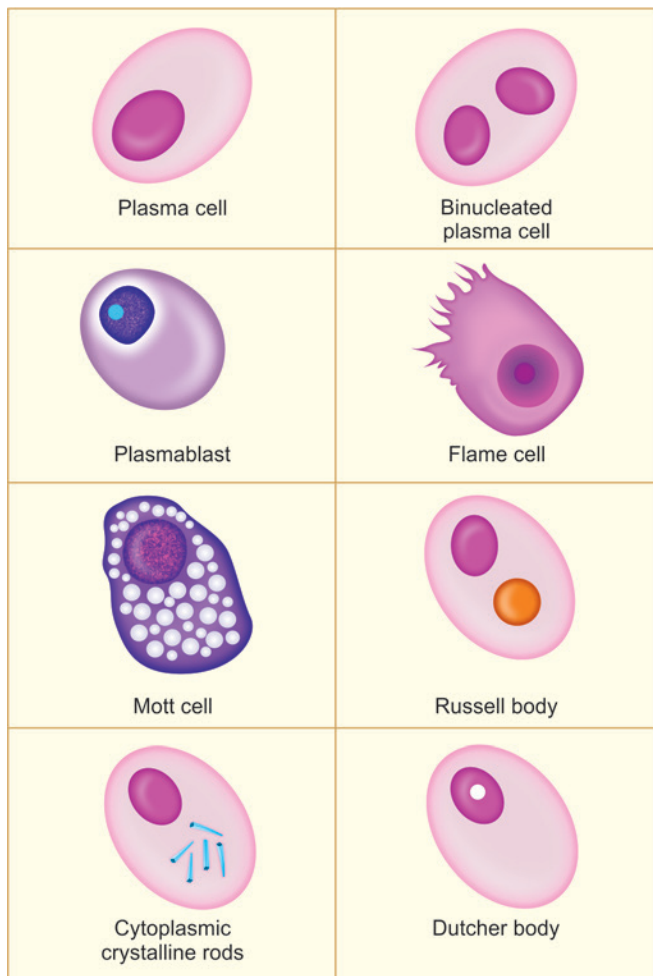


Fig. 11.28: Diagrammatic appearance of the various cells that can be seen in bone marrow in multiple myeloma

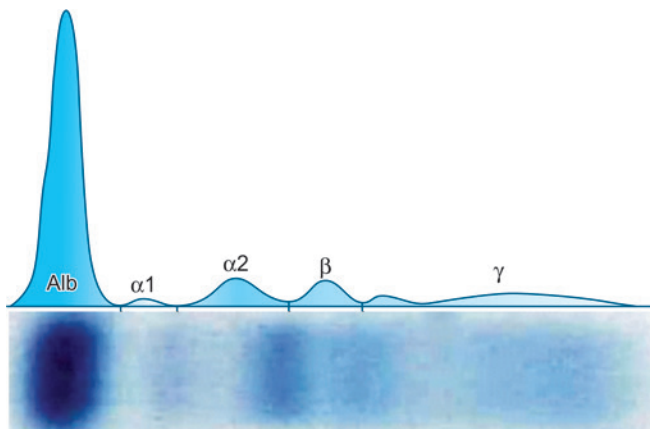
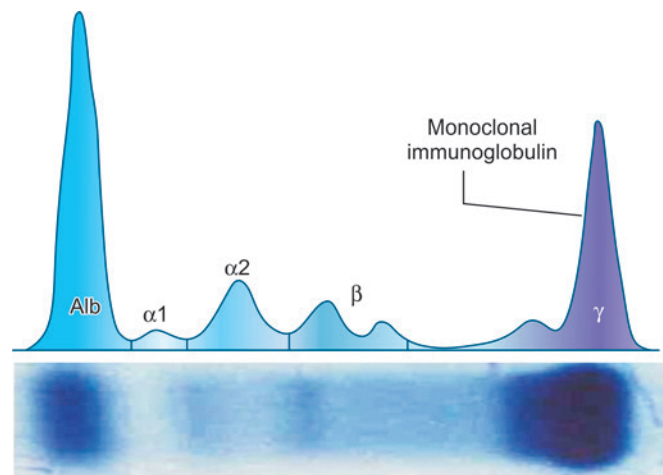


Fig. 11.29: Serum electrophoresis showing normal pattern



MM: involved bone shows multiple punched out lytic lesions.

Fig. 11.30: Serum electrophoresis showing monoclonal immunoglobulin ("M Band") in multiple myeloma



Fig. 11.31: Skull X-ray showing multiple punched-out lytic lesions

MM:

- Monoclonal gammopathy peak 50–60 years.
- Multiple lytic lesions in bones.
- Hypercalcemia.

Clinical Manifestations (Fig. 11.32)

Onset: Insidious.

Age and sex: Affects old age between 50 and 60 years with slight male preponderance.

The clinical features of multiple myeloma are

MM: Renal failure and sepsis are common causes of death.

1. Due to tumor cells causing bone lesions:

- **Resorption of bone:** This results in pathologic fractures, chronic bone pain and tenderness.

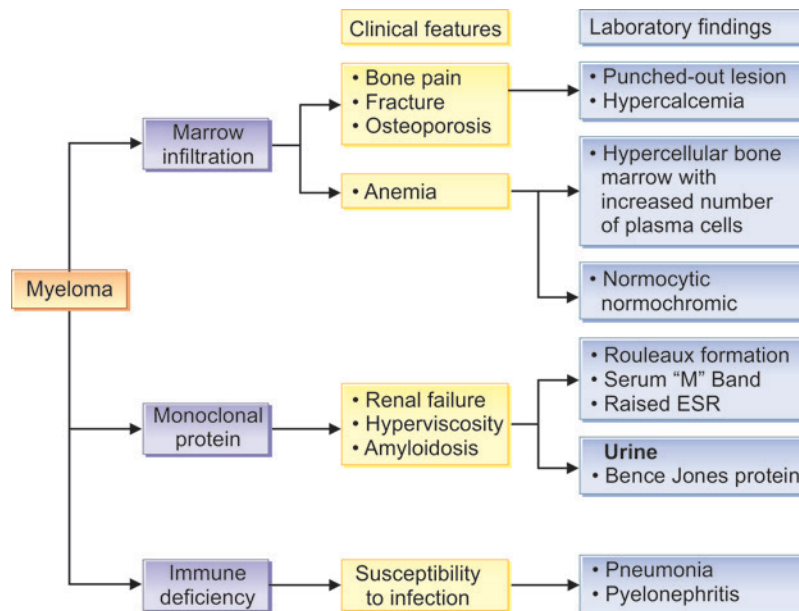


Fig. 11.32: Clinical features and laboratory findings in myeloma

Q. Write short note on radiological appearance of multiple myeloma

- **Compression:** Lesion in the vertebra may compress the spinal cord nerve root.
- **Hypercalcemia.**
- **Pallor:** Due to anemia and result in weakness and fatigue.

MM: Higher levels of serum β_2 microglobulin are associated with poor prognosis.

2. **Production of M-proteins (increased immunoglobulins):**
 - Bleeding tendency.
 - Coagulation abnormalities.
 - Amyloidosis of the AL type.
3. **Humoral immune deficiency:** Predisposes to recurrent bacterial infections.
4. **Renal disease:** Renal insufficiency, infections or nephrotic syndrome.

MM prognosis: Progressive course with poor prognosis.

Clinical Variants of Plasma Cell Myeloma

Asymptomatic (Smoldering) Plasma Cell Myeloma

Q. Write short essay/note on asymptomatic (smoldering) plasma cell myeloma

In asymptomatic plasma cell myeloma, the serum M protein is at myeloma level (more than 30 g/L) and/or 10% or more

clonal plasma cells in the bone marrow, but without any related organ or tissue damage. The patients are asymptomatic and carry a higher risk of progression to myeloma or related malignancy compared to monoclonal gammopathy of uncertain significance (MGUS).

Nonsecretory Myeloma

This is a form of multiple myeloma in which there is no M protein in either serum or urine. Renal involvement is less common than in myeloma.

PLASMACYTOMA

Localized proliferation forms a single discrete plasma cell tumor in bone (usually) or soft tissue.

- Solitary plasmacytoma of bone (osseous plasmacytoma).
- Extraosseous (extramedullary) plasmacytoma.

Extra-osseous plasmacytoma is usually found in the upper respiratory tract, especially in the nasal cavity and sinuses, nasopharynx and larynx.

IMMUNOGLOBULIN DEPOSITION DISEASE

Primary Amyloidosis

Plasma cell neoplasm secretes abnormal immunoglobulin light chains, which may get deposited in tissues and form a β -pleated sheet structure (AL amyloid).

MONOCLONAL GAMMOPATHY OF UNCERTAIN SIGNIFICANCE (MGUS)

- Presence of serum M protein concentration lower than 3 g/dL.
- Bone marrow clonal plasma cells less than 10% in an asymptomatic patient.
- **Etiology:** May represent an early stage of myeloma development.
- **Clinical manifestations:** Asymptomatic.

MGUS prognosis: Most of the patients remain stable.

LYMPHOID NEOPLASMS

CLASSIFICATION OF LYMPHOID NEOPLASMS (BOX 11.16)

Q. Write short essay/note on B-cell lymphoma.

Majority (80 to 85%) of lymphoid neoplasms are of B-cell origin and remaining of T-cell/NK cell type.

Lymphoid neoplasms: Most resemble some stage of B- or T-cell differentiation.

Lymphoid neoplasms: Second most common malignant tumor in HIV.

Lymphoid neoplasms: About 1/3rd arise from extranodal sites.

T-cell lymphoblastic lymphoma or Burkitt lymphoma usually seen in childhood.

WHO classification of lymphoid neoplasms depends on clinicopathological and immunological profile (Table 11.7) and has clinical and therapeutic importance.

FOLLICULAR LYMPHOMA

Q. Write short note on follicular lymphoma.

Composed of follicle center (germinal center) B cells of lymphoid follicles (centrocytes and centroblasts).

Molecular Pathogenesis

Q. Molecular pathogenesis of follicular lymphoma.

Follicular lymphoma is strongly associated with chromosomal translocations involving *BCL2*. It is characterized by a (14;18) translocation that juxtaposes the IGH locus on chromosome

BOX 11.16: WHO classification of the lymphoid neoplasms (2016)

I. PRECURSOR LYMPHOID NEOPLASMS

B lymphoblastic leukemia/lymphoma

T lymphoblastic leukemia/lymphoma

II. MATURE B-CELL NEOPLASMS

Chronic lymphocytic leukemia/small lymphocytic lymphoma

B cell prolymphocytic leukemia

Monoclonal B-cell lymphocytosis*

Splenic B-cell marginal zone lymphoma

Hairy cell leukemia

Lymphoplasmacytic lymphoma

Heavy chain disease

Plasma cell neoplasm

Follicular lymphoma

Mantle cell lymphoma

Diffuse large B-cell lymphoma

Burkitt lymphoma

III. MATURE T AND NK CELL NEOPLASMS

T-cell prolymphocytic leukemia

T-cell large granular lymphocytic leukemia

Mycosis fungoides

Sézary syndrome

Peripheral T-cell lymphoma, NOS

Angioimmunoblastic T-cell lymphoma

Anaplastic large cell lymphoma

Adult T-cell leukemia/lymphoma

Extranodal NK/T cell lymphoma, nasal type

IV. HODGKIN LYMPHOMA

Classical Hodgkin lymphoma

– Nodular sclerosis classical Hodgkin lymphoma

– Mixed cellularity classical Hodgkin lymphoma

– Lymphocyte-rich classical Hodgkin lymphoma

– Lymphocyte depleted classical Hodgkin lymphoma

Nodular lymphocyte predominance Hodgkin lymphoma

*Changes from the 2008 classification.

TABLE 11.7: Cell type and its antigens detected by monoclonal antibodies

Cell type	Antigen detected
T-cell	CD1, CD3, CD4, CD5, CD8
B-cell	CD10, CD19, CD20, CD21, CD23, CD79a
Monocyte or macrophage	CD11c, CD13, CD14, CD15, CD33, CD64
NK cell	CD16, CD56
Stem cell and progenitor cell	CD34
All leukocytes	CD45 (LCA)

Abbreviations: CD, cluster designation; NK, natural killer; LCA, leukocyte common antigen

14 and the *BCL2* locus on chromosome 18. This causes overexpression of *BCL2* which acts as antiapoptotic factor and promotes the survival of follicular lymphoma cells. Normal germinal centers contain numerous B-cells which undergo apoptosis. In follicular lymphoma there are no apoptotic cells. In about 90% of cases there are mutations in the *MLL2* gene (codes histone methyltransferase involved in epigenetics).

Morphology

FL: Arises from follicle center B-cells.

Gross

- Involves **lymph nodes**, spleen and bone marrow.
- **Architecture** of lymph node is **lost**; frequently infiltrate the perinodal tissue (Fig. 11.33).

Microscopy

FL: Centrocytes and centroblasts form poorly defined follicles.

- **Follicular** (nodular) growth pattern, **neoplastic follicles** are **poorly defined** (Fig. 11.34).

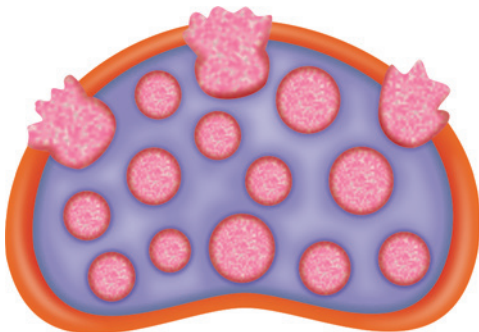


Fig. 11.33: Diagrammatic appearance of follicular lymphoma. Neoplastic follicles are seen in both the cortex and medulla and infiltration of the perinodal tissue

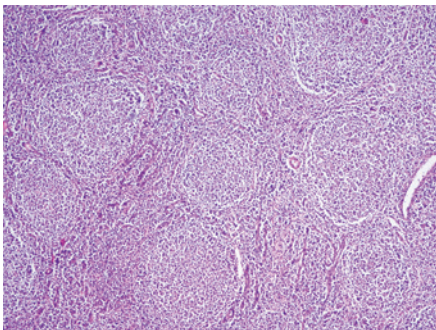


Fig. 11.34: Follicular lymphoma shows nodular aggregates of malignant lymphoid cells

Two types of B-cells.

- **Centrocytes** (small cleaved cells)
 - ◆ Cleaved nuclei
 - ◆ Inconspicuous nucleoli.
- **Centroblasts** (large noncleaved cells)
 - ◆ Round or oval nuclei with open nuclear (vesicular) chromatin.
 - ◆ Multiple (1 to 3) nucleoli.
 - ◆ Usually 3 times the size of lymphocyte.

FL: Grade ranges from 1 to 3. Grade 1 with less than 5 centroblasts/hpf and grade 3 with more than 15/hpf.

Immunophenotype

Expresses CD19, CD20 (pan-B cell markers), CD10 (CALLA), surface immunoglobulin and BCL2 protein.

Cytogenetics and Molecular Genetics

t (14; 18) (q32;q21), with IgH and BCL2 as partner genes and leads to constitutive overexpression of BCL2 protein.

FL: Peripheral blood smear may show lymphocytosis [less than $20 \times 10^9/L$ (20,000/cu mm)].

FL: Bone marrow involved in 85%.

Clinical Features

- Peak in **sixth and seventh decades**.
- **Generalized lymphadenopathy**.

FL: Prognosis indolent.

DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL)

Heterogeneous group of aggressive neoplasm of large B-cell with **diffuse** growth pattern.

Constitutes about 20 to 30% of NHL and 60% to 70% of aggressive lymphoid neoplasms.

DLBCL: Aggressive, diffuse large B-cell neoplasm.

Microscopy

DLBCL may involve lymph nodes or extranodal sites.

- **Loss of lymph node architecture** with **diffuse growth pattern**.
- **Neoplastic cells:**
 - **Large** round or oval cells, **4 to 5 times** of a **small lymphocyte**.
 - Moderate pale or basophilic cytoplasm.

- Nucleus equals or larger than the nucleus of a macrophage with different appearances.

Immunophenotype

- Express pan-B cell markers such as CD19, CD20, CD22 and CD79a.
- Also express germinal center markers like CD10 and BCL6.
- Negative for TdT.

Cytogenetics and Molecular Profile

- Translocation of BCL2 gene: t(14; 18) translocation
- Mutations of the BCL6 gene.

Clinical Features

- More common between 65 and 70 years of age.
- Slight male preponderance.
- Rapidly enlarging mass at a single or multiple nodal or extranodal sites.

DLBCL: Prognosis aggressive and rapidly fatal if untreated.

BURKITT LYMPHOMA

Q. Write short note on Burkitt lymphoma.

- Highly aggressive B cell neoplasm, often presents as extranodal lymphoma or as an acute leukemia.
- Composed of medium-sized, monomorphic lymphoid cells with basophilic vacuolated cytoplasm.

Clinical Variants

- Endemic (African) Burkitt lymphoma (BL):
 - Occurs in Africa, affects children and adolescents.
 - Associated with Epstein-Barr virus infection and malaria.
 - Usually involves the jaw and present as a mandibular mass.
- Sporadic (nonendemic) BL:
 - Occurs in children or young adults.
 - Abdominal mass and involves ileocecum and peritoneum.
- Immunodeficiency-associated (HIV) BL:
 - Involves lymph nodes and bone marrow.

BL: Aggressive B-cell lymphoma, 3 clinical variants. Endemic: involves jaw and associated with EBV.

Microscopy

BL: Medium sized B-cells. Starry sky pattern.

- Burkitt lymphomas, irrespective of the categories, are histologically similar.
- Lymph node shows loss of architecture.
- Involved tissues show diffuse infiltrate of monotonous medium-sized lymphoid cells (Figs 11.35 and 11.36).
- Appearance of neoplastic lymphoid cells:
 - Medium-sized cells.
 - Round or oval nuclei having clumped coarse chromatin with several (2–5) nucleoli.
 - Moderate amount of deeply basophilic cytoplasm, multiple, small, round lipid (clear) vacuoles which stain positive with oil red O.
 - Numerous mitotic figures.
- Starry sky pattern: Tumor cells undergo apoptosis and nuclear remnants of these apoptotic cells are phagocytosed and cleared by benign macrophages. These macrophages in the background of lymphoid cells creates “starry sky” appearance (Figs 11.35 and 11.36).

Immunophenotype

- Express surface IgM, monotypic κ or λ light chain.
- Positive for common B-cell antigens (CD19, CD20, and CD22).
- Positive for CD10 and BCL6.
- BCL2 negative.

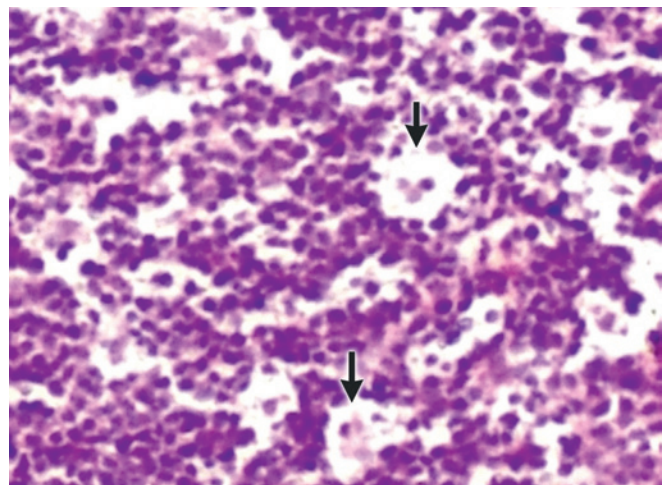


Fig. 11.35: Burkitt lymphoma composed of medium-sized lymphoid cells admixed with benign macrophages giving a “starry sky” appearance

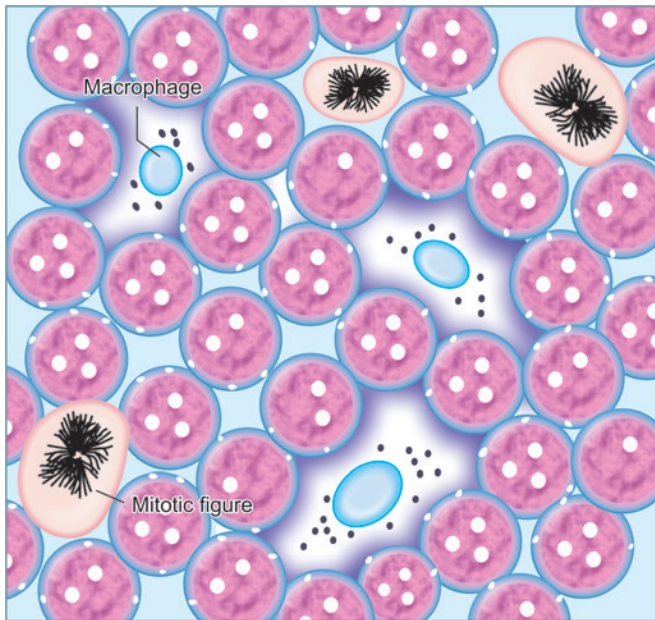


Fig. 11.36: Diagrammatic appearance of Burkitt lymphoma composed of medium-sized lymphoid cells admixed with benign macrophages giving a “starry sky” appearance

Cytogenetic and Molecular Genetic Features (Fig. 11.37)

Translocations of *c-MYC* gene

BL: Translocation of *c-MYC* gene.

- *MYC* (*c-MYC*) is a **proto-oncogene** on chromosome 8.
- **Most common translocation t (8:14) (q24; q32).**
- **Translocations of *c-MYC* gene, converts proto-oncogene into *MYC* oncogene, which leads to overexpression of**

***MYC* protein (oncoprotein).** This causes **uncontrolled cell proliferation** and stimulation of apoptosis.

BL: Prognosis—very aggressive but responds well chemotherapy.

- **Mutations inactivate p53.**
- **Poor prognostic factors:**
 - Involvement of blood, bone marrow and central nervous system.
 - Bulk of the disease-unresected tumor of more than 10 cm in diameter.
 - High serum LDH levels.
 - Presence of residual disease after excision.

MATURE T-CELL AND NK CELL NEOPLASMS

Peripheral T cell tumors constitute less than 15% of non-Hodgkin lymphomas. NK cell tumors are very rare.

Peripheral T Cell Lymphoma (PTCL), NOS

Mainly involves lymph node.

Microscopy

PTCL: Clinical features

- Fifth to seventh decade.
- Generalized lymphadenopathy.
- **Lymph node with effacement of the normal architecture.**
- **Paracortical or diffuse infiltration by neoplastic T-cells.**
- **Neoplastic T-cells.**

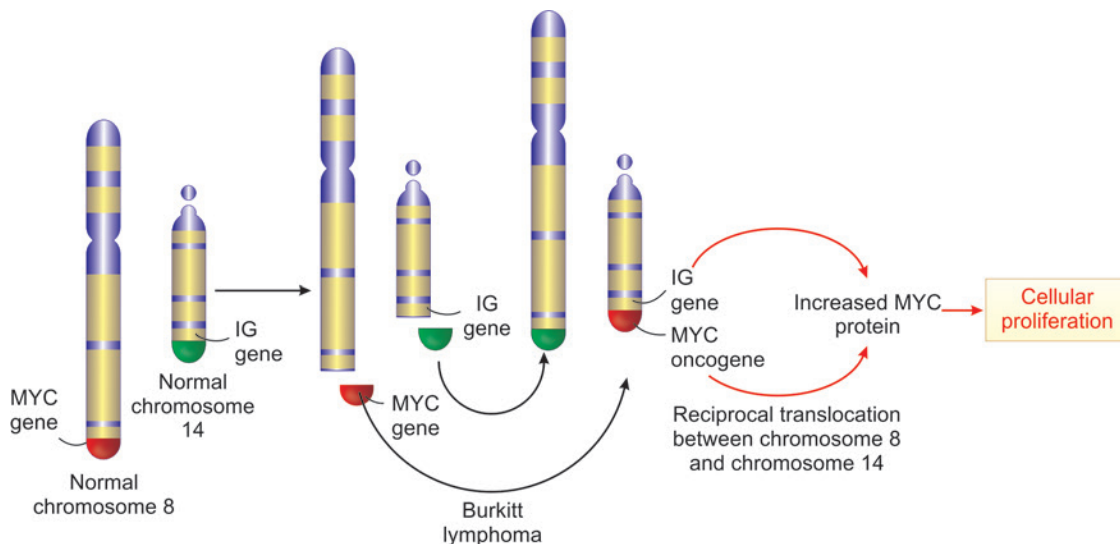


Fig. 11.37: Chromosomal translocation and activated MYC oncogene in Burkitt lymphoma

- **Small, intermediate to large cells** with sparse or abundant; clear, eosinophilic or basophilic.
- **Vesicular or hyperchromatic nuclei, prominent nucleoli.**

Immunophenotype

PTCL prognosis: Highly aggressive with a poor response to therapy.

- Lack TdT (expressed by immature T-cells).
- Express pan-T cell-CD2, C3, CD5 and either $\alpha\beta$ or $\gamma\delta$ T cell receptors (TCR).

Mycosis Fungoides

Mycosis fungoides and Sézary syndrome: T-cell neoplasms with skin involvement.

- **Cutaneous T-cell lymphoma.**
- Lymphoid cells with **irregular nuclear outlines.**
- Limited to **skin.**

Age: Most are adults or elderly.

Microscopy

Mycosis fungoides has three stages:

1. Patch stage
 2. Plaque stage
 3. Tumor stage.
- **Epidermis (epidermotropism) and upper dermis** is infiltrated by neoplastic T-cells.
 - Groups of neoplastic cells in the epidermis—**Pautrier's microabscess.**
 - Tumor cells have **convoluted (cerebriform) nuclear contours.**

Immunophenotype: Express pan-T-CD2+, CD3+ and CD5.

Sézary Syndrome

Rare disease and is defined by the triad namely:

1. **Widespread exfoliative erythroderma**
2. **Generalized lymphadenopathy**
3. **Presence of characteristic Sézary cells** in the skin, lymph nodes and peripheral blood.

Sézary cells are neoplastic T-cells with cerebriform nuclei.

Immunophenotype: Tumor cells express-CD2⁺, CD3⁺ and CD5⁺.

Prognosis

Aggressive disease and most die of opportunistic infections.

HODGKIN LYMPHOMAS

DEFINITION

Hodgkin lymphoma synonym: Hodgkin disease.

HL: Malignant lymphoid neoplasms with following characteristics:

- **Minority (1–3%) of specific neoplastic cells** (Hodgkin cells and **Reed-Sternberg cells**).
- **Majority background of reactive non-neoplastic cells.**
- Usually involves **lymph nodes.**
- Majority occurs in **young adults.**

CLASSIFICATION (BOX 11.17)

Q. Classify Hodgkin lymphoma.

Hodgkin lymphoma (HL) is broadly divided into **two types**, which differ in clinical features, behavior, morphology and immunophenotype.

Cell of Origin and Immunophenotype

- **Classical Hodgkin lymphoma**
 - Cell of origin: **Germinal center or post-germinal center B cell**
 - Immunophenotype: CD15 and CD 30 **positive.**

BOX 11.17: WHO classification (2016) of Hodgkin lymphoma

- **Classical Hodgkin lymphoma (CHL)**
 - **Nodular sclerosis classical Hodgkin lymphoma (NSCHL)**
 - **Mixed cellularity classical Hodgkin lymphoma (MCCHL)**
 - **Lymphocyte-rich classical Hodgkin lymphoma (LRCHL)**
 - **Lymphocyte depleted classical Hodgkin lymphoma (LDCHL)**
- **Nodular lymphocyte predominant Hodgkin lymphoma (NLPHL)**

Classical HL: CD15⁺ and CD30⁺,
NLPHL: CD15[−], CD 30[−], CD20⁺, and CD 45⁺.

- **Nodular lymphocyte predominant Hodgkin lymphoma**
 - Cell of origin: **Germinal center B cell at the centroblastic stage** of differentiation.
 - Immunophenotype: **CD15 and CD30 negative**.

HL is mainly divided into:

1. Classical and
2. Nodular lymphocytic Hodgkin lymphoma.

MORPHOLOGY OF NEOPLASTIC CELLS

Q. Write short note on RS cell and its variants.

Reed-Sternberg (RS) Cells are neoplastic cells (Fig. 11.38) pathognomonic of Hodgkin lymphoma.

Appearance and description of diagnostic Reed-Sternberg cells and its variants are shown in Figure 11.39.

Various types of cells found in Hodgkin lymphoma are listed in Table 11.8.

CLASSICAL HODGKIN LYMPHOMA

Q. Describe the gross and microscopic features of all subtypes of Hodgkin lymphoma. Compare their prognosis.

Classical Hodgkin lymphoma (CHL) account for **95%** of Hodgkin lymphomas and has 4 subtypes.

Nodular Sclerosis Classical Hodgkin Lymphoma

Q. Write short note on nodular sclerosis HL.

Nodular sclerosis is the most common subtype of CHL. Lacunar cells are commonly seen.

TABLE 11.8: Types of cells found in Hodgkin lymphoma

Non-neoplastic cells	Neoplastic cells
• Reactive lymphocytes	• Reed-Sternberg cells (classical)
• Macrophages/histiocytes	• Variants
– Granulocytes	– Mononuclear
– Eosinophils	– Lacunar
– Neutrophils	– Mummified
• Plasma cells	– Anaplastic/pleomorphic
	– Lymphocyte predominant (LP) cell/popcorn

HL: Majority are non-neoplastic cells and minority are neoplastic cells.

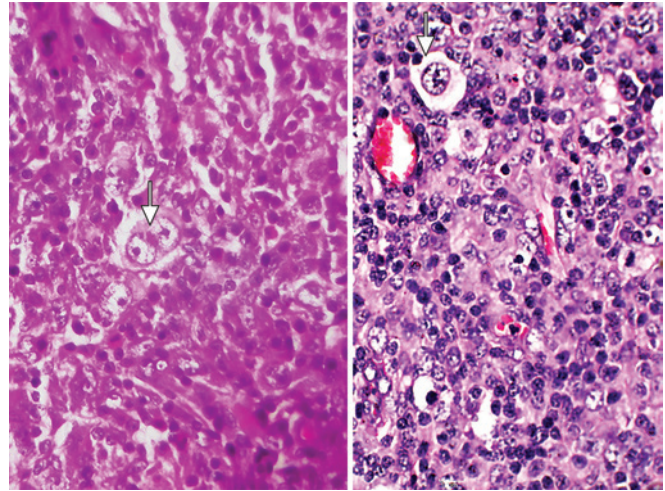


Fig. 11.38: Microscopic appearance of Hodgkin lymphoma showing RS cells (short arrow and inset) and Hodgkin cells (long arrow) within the background of mixed population of reactive cells

Subtype of CHL characterized by **collagen bands** that surround **nodules** and have **lacunar cell variant** of Reed-Sternberg cells.

- Most common: **40%–70%** of cases.
- Most between **20 and 30 years of age** with **equal frequency** in males and females.
- **Rarely associated with EBV.**
- Involves **mediastinal lymph nodes**.

Microscopy of NSCHL (Fig. 11.40)





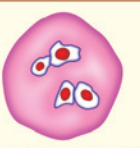

NSCHL

- Nodules separated by broad bands of collagen
- CD15+, CD30+, EBV-ve and CD 45-ve.
- **Loss of lymph node architecture.**
- **Sclerosis and nodules:** Broad collagen bands (sclerosis) divide the lymphoid tissue into **nodules of varying sizes and shapes**.
- **Presence of lacunar cell.**
- **Background:** small T lymphocytes, eosinophils, plasma cells, and macrophages.

NSCHL prognosis: Better than other types of CHL, with a cure rate of 80%–85%.

Immunophenotype

- RS cells are **CD15+ and CD30+**; **CD45- and T cell markers negative**.
- **EBV negative.**

DESCRIPTION	APPEARANCE
Large cells (20–60 μm in diameter) Abundant eosinophilic cytoplasm Nucleus: Typically two large nuclei—"mirror image" Prominent eosinophilic nucleolus surrounded by a halo	 Classic RS cell
Single, large, round nucleus with a large eosinophilic inclusion-like nucleolus May be seen in any subtypes of CHL	 Mononuclear cell variant (Hodgkin cell)
Abundant, lightly acidophilic or water-clear cytoplasm Large folded or multilobed nucleus One or more prominent eosinophilic nucleoli Seen in nodular sclerosis of CHL	 Lacunar cell variant
Dark eosinophilic cytoplasm with pyknotic nuclei Strongly indicate HL but not diagnostic	 Mummified cell variant
Highly pleomorphic cells Large bizarre hyperchromatic nuclei Commonly found in lymphocyte-depleted CHL	 Anaplastic/pleomorphic variant
Abundant pale cytoplasm with popcorn nucleus Specific to the nodular lymphocyte predominance type of HL	 Lymphocyte predominant cell (LP cell)/popcorn cell

Classical RS cell is binucleated with owl-eyed nuclei having mirror image appearance.

Lacunar cell has clear cytoplasm and seen in nodular sclerosis CHL.

LP/popcorn cell is seen in nodular lymphocyte predominant HL.

Fig. 11.39: Diagrammatic appearances and characteristic features of Reed-Sternberg cells and its variants

Mixed Cellularity Classical Hodgkin Lymphoma (MCCHL)

Q. Write short note on mixed cellularity HL.

- **Second common subtype:** 20%–25% of cases.
- **More common in males**
- **Strongly associated with EBV.**
- **Older age, with systemic symptoms** (such as night sweats and weight loss) and **advanced tumor stage.**
- Involves **peripheral lymph nodes.**

MCCHL: Scattered classical RS cells and mixed inflammatory background, CD15+, CD30+ and EBV+.

Microscopy of MCCHL (Fig. 11.41)

- Lymph node architecture obliterated.
- **Plenty of Reed-Sternberg cells and Hodgkin cells.**
- **Background:** Small lymphocytes, eosinophils (sometimes numerous), neutrophils, plasma cells and benign macrophages (histiocytes).

MCCHL prognosis: Very good.

Immunophenotype

RS cells are CD15+, CD30+ and EBV+ (about 70%).

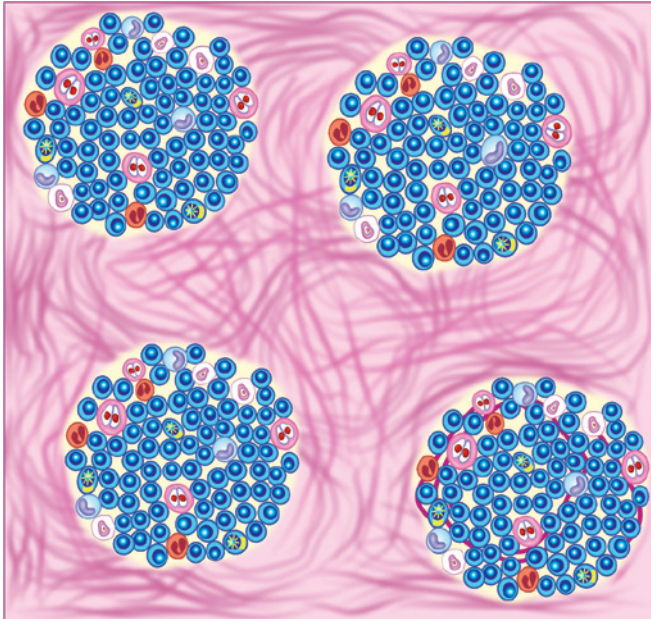


Fig. 11.40: Nodular sclerosis classical Hodgkin lymphoma with nodules separated by bands of collagen. Also seen are lacunar cells and RS cells in each nodule within the background of lymphocytes, eosinophils, plasma cells and macrophages

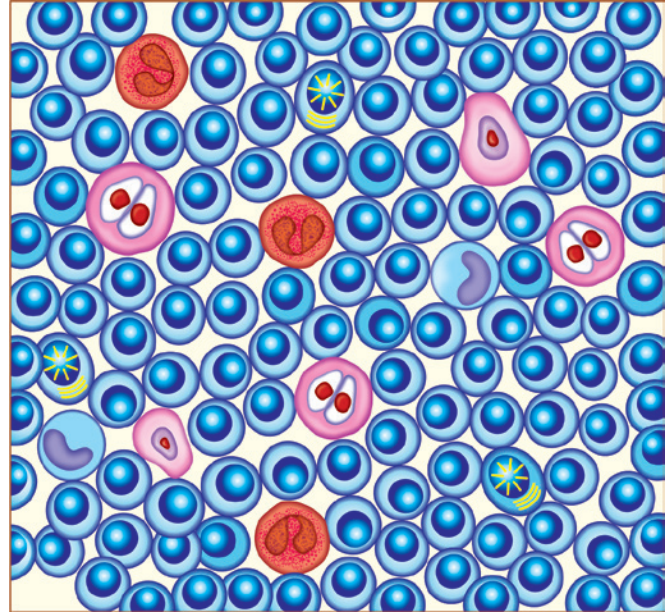


Fig. 11.41: Mixed cellularity classical Hodgkin lymphoma with classical RS cells, Hodgkin cells in the background of mixed cellular population consisting of lymphocytes, eosinophils, plasma cells and macrophages

Lymphocyte-rich Classical Hodgkin Lymphoma (LRCHL)

- Subtype of classical Hodgkin lymphoma with **scattered Hodgkin and RS cells**.
- **Uncommon**—about 5% of classical HL.
- More in **elderly** patients, associated with EBV in **40%** of cases.
- Involves **peripheral lymph nodes**.

Microscopy of LRCHL (Fig. 11.42)

LRCHL:

- Uncommon.
- Few RS cells.
- Abundant lymphocytes.
- CD15⁺, CD30⁺, CD45⁺ and CD20⁺.
- **Growth patterns:** May show two patterns.
 - **Nodular**—common
 - **Diffuse**—rare
- **Only few Reed-Sternberg cells and Hodgkin cells.**
- **Background:** Abundant reactive small lymphocytes.

Immunophenotype

CD45⁺, CD20⁺, CD15⁺ and CD30⁺.

LRCHL prognosis: Good to excellent prognosis.

Lymphocyte-depleted Classical Hodgkin Lymphoma (LDCHL)

LDCHL:

- Rarest.
- Paucity of lymphocytes.
- Plenty of RS cells.
- CD15⁺, CD30⁺; majority are EBV⁺.

Subtype of classical Hodgkin lymphoma **rich in Hodgkin and RS cells** in a background depleted in non-neoplastic lymphocytes.

- **Rarest**—less than 5% of cases
- Predominantly in **older, HIV-positive** patients, often **EBV-associated** (over 90%)
- Predominantly **retroperitoneal lymph nodes, abdominal organs and bone marrow**.

Microscopy of LDCHL (Fig. 11.43)

- Paucity of lymphocytes.
- Plenty of RS cells or their **anaplastic/pleomorphic variants**.
- Histological types
 - **Reticular:** Numerous Hodgkin and RS cells with depletion of lymphocytes.
 - **Diffuse sclerosis/fibrosis:** Hypocellular infiltrate containing bizarre RS cells with fine fibrosis.

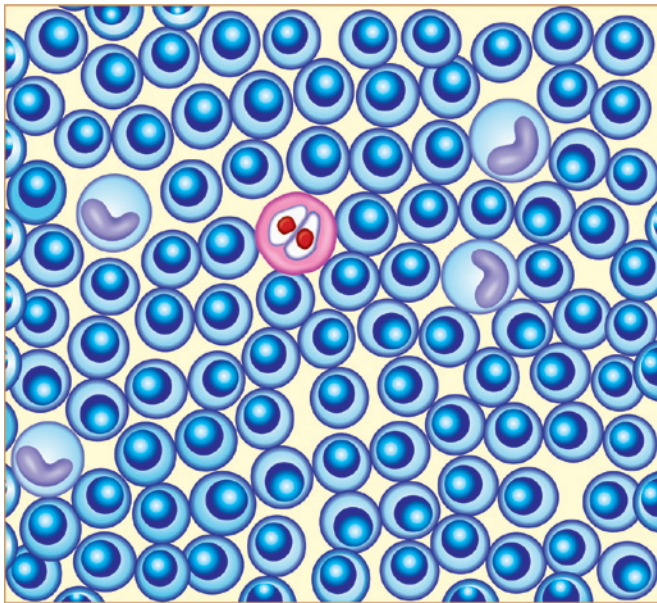


Fig. 11.42: Lymphocyte-rich classical Hodgkin lymphoma. One RS cell is seen in a background of many small lymphocytes and few histiocytes

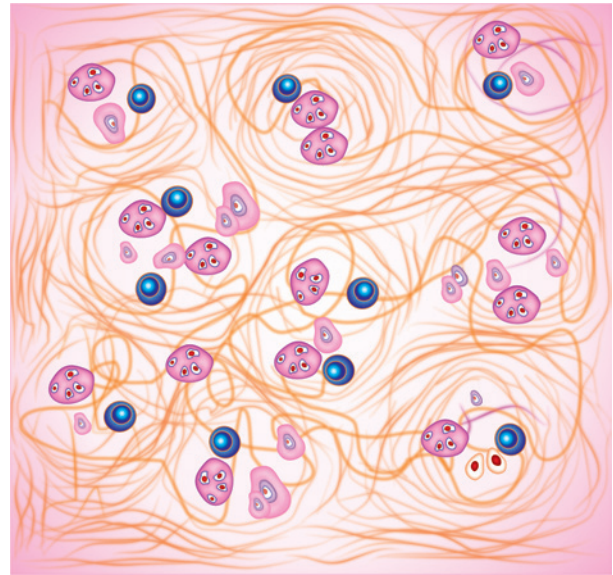


Fig. 11.43: Lymphocyte-depleted classical Hodgkin lymphoma with the pleomorphic variant of RS cells surrounded by fibrous tissue

Immunophenotype

RS cells are CD15+, CD30+; majority are EBV+.

LDCHL prognosis: Outcome less favorable than with other subtypes.

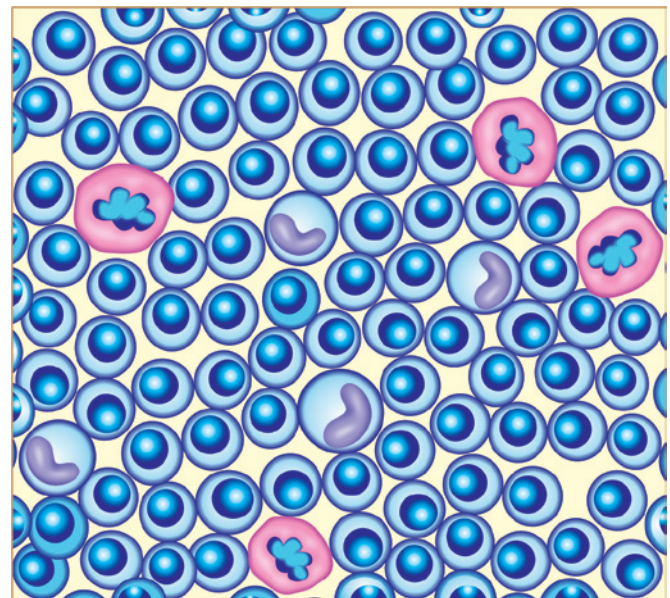
NODULAR LYMPHOCYTE PREDOMINANT HODGKIN LYMPHOMA (NLPHL)

- **Uncommon**—5% of all Hodgkin lymphomas.
- **Not associated with EBV.**
- Majority males, usually **30–50 year** of age.
- Involves mainly **cervical or axillary lymph nodes**.

Microscopy of NLPHL (Fig. 11.44)

NLPHL:

- Uncommon.
- Abundant lymphocytes.
- LP cells.
- No Hodgkin/RS cells.
- CD20+, CD45+ and CD15–, C30– and EB negative.
- Loss of lymph node architecture.
- Nodular and/or diffuse infiltrate of **abundant small lymphocytes** with histiocytes and **scattered LP cells**.
- **Lymphocyte predominant cells (LP cells)**/"popcorn" cells (Fig. 11.39):
 - Specific to NLPHL.



NLPHL prognosis: More likely to recur than the classical subtypes, but the prognosis is very good.

Fig. 11.44: Nodular lymphocyte predominant Hodgkin lymphoma with 'popcorn' cells in a background of reactive lymphocytes and few macrophages

- Large with relatively abundant, pale cytoplasm.
- Single large delicate **multilobulated nucleus or folded nuclei** resembling bubbly outlines of popcorn kernels.
- One or more **inconspicuous nucleoli**.
- **Hodgkin and RS cells are not found.**

Immunophenotype

LP cell are CD20+, CD 45+ and CD15-, C30- and EBV-ve. Express BCL6.

ETIOLOGY AND PATHOGENESIS OF HODGKIN LYMPHOMA

- **EBV:** Previous EBV infection (infectious mononucleosis) ↑ risk of HL.
- **Genetic factors:** HLA-B18 higher in HL.
- **Immune status:** HL more frequent in immunocompromised patients and autoimmune diseases (e.g. rheumatoid arthritis).

Pathogenesis (Fig. 11.45)

- **EBV and HL:** HL is associated with EBV infection.
- **Activation of nuclear factor (NF-κB) common event** in classical HL → rescue germinal center B-cells from apoptosis → **produces Reed-Sternberg cells.**
- **Accumulation of reactive cells** in response to *cytokines* (such as IL-5, IL-6 and TGF-β) and *chemokines* secreted by Reed-Sternberg cells.

LABORATORY FINDINGS

Peripheral smear:

- **RBCs:** Normocytic normochromic anemia.
- **WBCs:** Leukocytosis occurs in 1/3rd of the patients. Eosinophilia is frequent.
- **Platelets:** Normal or increased.

ESR: raised.

Bone marrow: Involved in the later stages.

Fine Needle Aspiration Cytology (FNAC)

RS cells/its variants against a background of inflammatory cells (depending on the subtype).

Spread

- **Mainly by contiguity**
- First nodal disease → then splenic disease, hepatic disease → and finally marrow involvement and extranodal disease.

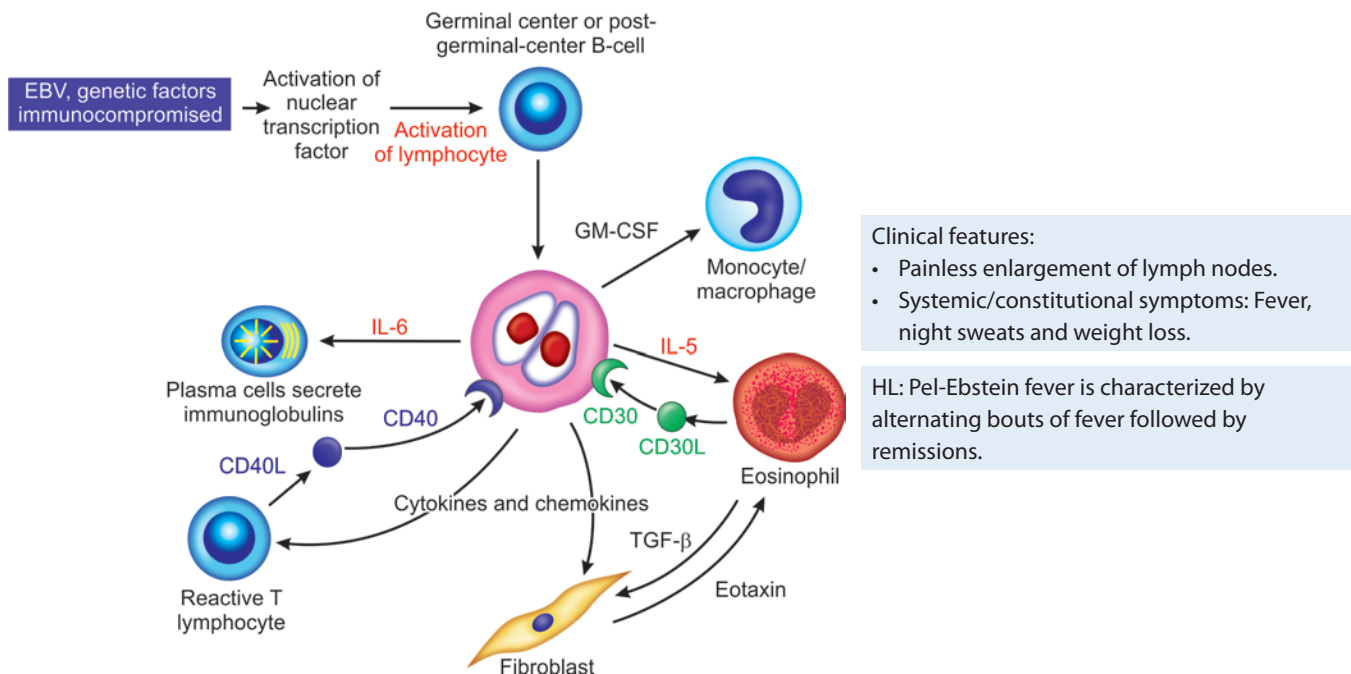


Fig. 11.45: Pathogenetic mechanism and interaction of various cell types in Hodgkin lymphoma

STAGING OF HODGKIN LYMPHOMA (TABLE 11.9)

TABLE 11.9: Clinical staging of Hodgkin lymphomas (Cotswold revision of Ann Arbor staging classification)

Stage	Definition
I	Involvement of a single lymph node region or lymphoid structure (e.g. spleen, Waldeyer ring, thymus)
II	Involvement of two or more lymph node regions on the same side of the diaphragm (the mediastinum is a single site; hilar lymph nodes are lateralized); the number of anatomic sites should be indicated by suffix (e.g. II ₃)
III	Involvement of lymph node regions or structures on both sides of the diaphragm
	III ₁ With or without splenic, hilar, celiac or portal nodes III ₂ With para-aortic, iliac or mesenteric nodes
IV	Involvement of extranodal site(s) beyond those designated E

E, involvement of a single extranodal site, or contiguous or proximal to known nodal site of disease

TABLE 11.10: Differences between Hodgkin and non-Hodgkin lymphomas

Sl. No.	Characteristics	Hodgkin lymphoma	Non-Hodgkin lymphoma
1.	Site of involvement	Arises in a single node or chain of nodes (cervical, mediastinal, para-aortic)	Mainly involves multiple peripheral nodes
2.	Pattern of spread	Orderly spread by contiguity in a predictable fashion	Noncontiguous spread in an unpredictable fashion
3.	Mesenteric nodes and Waldeyer ring	Rarely involved	Commonly involved
4.	Extranodal involvement	Uncommon	Common
5.	Characteristic of neoplastic cells	Neoplastic cells—Hodgkin or Reed-Sternberg cells form minor tumor cell mass (1–5%)	Neoplastic cells form the major tumor cell mass

TABLE 11.11: Types of Langerhans cell histiocytosis

Terminology/site	Involved tissue/ organ	Clinical features
Eosinophilic granuloma Localized to a single site/solitary (unifocal)	Bone and adjacent soft issue (skull, femur, vertebra, pelvic bones and ribs). Less commonly lymph nodes	Usually seen in older children or adults. Presents with lytic bone lesion
Hand-Schüller-Christian disease Multiple sites within a single system (multifocal unisystem)	Usually bone and soft tissue	Usually seen in young children. Multiple destructive bone lesions with adjacent soft tissue masses
Letterer-Siwe disease Disseminated and multisystemic disease (multifocal multisystem disease)	Skin, bone, liver, spleen and bone marrow	Usually seen in infants. Present with fever, cytopenias, skin and bone lesions and hepatosplenomegaly

DIFFERENCES BETWEEN HODGKIN LYMPHOMA AND NON-HODGKIN LYMPHOMA

HL differs from NHL in several respects and their main differences are shown in Table 11.10.

Q. List the differences between HL and NHL.

HL: Extranodal involvement uncommon.

LANGERHANS CELL HISTIOCYTOSIS/ HISTIOCYTOSIS X

INTRODUCTION

- Histiocytic and dendritic cell neoplasms.
- Clonal proliferative disorder arising from Langerhans cells.

- Langerhans cell histiocytosis (LCH) spectrum ranges from unifocal to multifocal and unisystem to multisystem disease.
- **Immunological markers:** Express CD1a, langerin and S-100 protein.

Langerhans cell contains pathognomonic Birbeck granules.

MORPHOLOGY

- **Light microscopy:** The characteristic feature is proliferations of **Langerhans cells**.
 - These are large cells 10–15 μm in diameter, moderate slightly eosinophilic **cytoplasm** folded, indented, grooved or lobulated nucleus having fine chromatin.
 - **Background:** Mixed background of eosinophils, histiocytes (mononuclear and multinuclear), neutrophils and small lymphocytes.
- **Electron microscopy:** Langerhans cell contains **pathognomonic Birbeck granules—tennis racket-like shape, with a zipper-like appearance**.

LABORATORY FINDINGS

- **Peripheral blood:** Pancytopenia (anemia, neutropenia and thrombocytopenia).
- **Bone marrow:** Extensive infiltration by histiocytes.

Prognosis: Depends on the age at presentation, extent of disease and rate of progression.

Groups: Depending on the site involved and distribution of lesion, LCH can be divided into three groups (Table 11.12).

Disorders of Hemostasis

DISORDERS OF PRIMARY HEMOSTASIS

NORMAL HEMOSTASIS

- Hemostasis is the **body's response to vascular damage/injury**.
- Includes several sequences of events at the site of vascular injury. They are as follows:

Primary Hemostatic Plug

Platelet sequence in hemostasis: Platelet adhesion → release of granule contents → platelet aggregation → primary (temporary) hemostatic plug → activation of coagulation system → fibrin → secondary (permanent) hemostatic plug.

Platelet adhere to subendothelial structures at the site of injury. The **platelets change their shape** and **release granule contents**. The released contents cause platelet aggregation and form **primary hemostatic plug**.

Secondary Hemostatic Plug

Exposure of **tissue factor** at the site of vascular injury **activates the extrinsic coagulation system**. The **fibrin formed** develops into a **secondary hemostatic plug**.

Terminology used in Bleeding Disorders

Petechiae: They are small (1–2 mm in diameter), red to purple hemorrhagic spots in the skin, mucous membranes or serosal surfaces. They result from blood

leaking through intact endothelial lining of capillaries. They are most **commonly found with low platelet counts (thrombocytopenia)** or **defective platelet function**.

Q. Define and classify purpura

- **Purpura:** The term purpura means purple. They are slightly larger (>3 mm) than petechiae. The causes are **thrombocytopenia, increased vascular fragility and vasculitis**.
 - **Purpura may be classified as non-thrombocytopenic (vascular) purpura** (refer Box 12.2) and **thrombocytopenic** (refer Box 12.3).
- **Ecchymoses:** They are larger (>1–2 cm) and result from blood escaping through endothelium into intact subcutaneous tissue. RBCs in the lesions are degraded and the released hemoglobin gives rise to red-blue color. The pigment from heme is converted into biliverdin and then to bilirubin (blue-green color) and iron from red cells forms hemosiderin (golden-brown color). These changes are responsible for the characteristic color changes in ecchymoses.
- **Hematoma:** It is formed when blood leaks from a vessel and collects within a tissue. It is blue or purple and slightly raised.

CLASSIFICATION OF HEMOSTATIC DISORDERS (BOX 12.1)

Q. Classify bleeding disorders.

1. **Bleeding disorders (hemorrhagic disorders/hemorrhagic diathesis):** Bleeding disorders have an **abnormal tendency to bleed** (hemorrhage) due to failure of hemostasis.
2. **Thrombotic disorders:** They cause **thrombus formation**.

BOX 12.1: Classification of disorders of hemostasis**Bleeding disorders**

- **Disorders of primary hemostasis**
 - **Vessel wall abnormalities**
 - ◆ Congenital, e.g. Ehlers–Danlos syndrome
 - ◆ Acquired, e.g. Henoch–Schönlein purpura
 - **Platelet abnormalities**
 - ◆ Quantitative: Thrombocytopenia (e.g. ITP, drug-induced, congenital)
 - ◆ Qualitative: Platelet function disorders
 - ◇ Inherited, e.g. Glanzmann thrombasthenia, Wiskott–Aldrich syndrome, Bernard Soulier syndrome
 - ◇ Acquired, e.g. uremia, drugs
- **Disorders of coagulation system (disorders of secondary hemostasis)**
 - ◆ **Congenital:** Hemophilia A, B; von Willebrand disease; other coagulation factor deficiencies [XI, VII, II, V, X]
 - ◆ **Acquired:** Vitamin K deficiency, liver disease, disseminated intravascular coagulation

Thrombotic disorders

- **Inherited**
 - Deficiency of antithrombotic factors: Antithrombin III deficiency, protein C deficiency, protein S deficiency
 - Increased prothrombotic factors: Activated protein C (APC) resistance (Factor V mutation/factor V Leiden)
 - Prothrombin (G20210A mutation)
- **Acquired:** Fibrinolytic system defects

Hemostatic disorders are broadly classified as bleeding disorders and thrombotic disorders.

Bleeding disorders may be due to:

- Diseases of blood vessels
- Platelet disorders
- Coagulation disorders.

Vascular purpuras are also known as nonthrombocytopenic purpuras.

BLEEDING DISORDERS CAUSED BY VESSEL WALL ABNORMALITIES

Vascular purpura (nonthrombocytopenic purpura) is group of **disorders of blood vessels** that results in **bleeding**. They should be distinguished from bleeding disorders due to abnormalities of platelets.

Classification of bleeding disorders caused by vessel wall abnormalities are presented in Box 12.2.

BLEEDING DISORDERS DUE TO ABNORMALITIES OF PLATELET

Classification of Platelet Disorders (Box 12.3)

BOX 12.2: Classification of bleeding disorders caused by vessel wall abnormalities**Acquired disorders**

1. Due to decreased amount of connective tissue
 - Senile purpura
 - Scurvy
 - Cushing syndrome and steroid therapy
2. Due to vasculitis
 - Henoch–Schönlein purpura
 - Infections
 - Drug reactions
3. Associated with plasma cell neoplasms
 - Amyloidosis
4. Miscellaneous
 - Simple easy bruising

Congenital/inherited disorders

- Hereditary hemorrhagic telangiectasia
- Ehlers–Danlos syndrome
- Marfan syndrome

Senile purpura is due to vessel instability.

Henoch–Schönlein purpura is characterized by hypersensitivity vasculitis and palpable purpura.

BOX 12.3: Classification of platelet disorders**Quantitative platelet disorders**

- **Thrombocytopenia**
 - Decreased platelet survival
 - Decreased production
 - Sequestration
 - Dilutional
- **Thrombocytosis**

Qualitative platelet disorders

- **Hereditary**
 - Defective adhesion of platelets
 - Disorders of platelet secretion
 - Defective platelet aggregation
- **Acquired**

THROMBOCYTOPENIA

- Decrease in the **platelet count** below the lower limit of **150,000/cu mm** ($150 \times 10^9/L$).

Clinical Features of Thrombocytopenia

- Cutaneous bleeding appears as pinpoint hemorrhages (petechiae) and ecchymoses.
- Mucosal bleeding.
- Intracranial bleed (subarachnoid and intracerebral hemorrhage) rare but serious.

Petechiae are pinpoint hemorrhages seen only with thrombocytopenia.

Severity of Bleeding

- **Post-traumatic bleeding**—when the platelet count is 20,000–50,000/cu mm
- **Spontaneous bleeding**—when the platelet count falls below 20,000/cu mm
- **Intracranial bleeding**—when platelet count is <10,000/cu mm.

Intracranial bleeding occurs when platelet count is <10,000/cu mm.

Causes of Thrombocytopenia (Box 12.4)

Q. Write short essay/note on causes of thrombocytopenia

BOX 12.4: Causes of thrombocytopenia

1. Decreased platelet survival

- Immunological destruction
 - Primary autoimmune
 - ◆ Acute immune thrombocytopenic purpura
 - Secondary autoimmune
 - ◆ Systemic lupus erythematosus, B-cell lymphomas
 - ◆ Chronic immune thrombocytopenic purpura
 - ◆ Alloimmune: Post-transfusion or pregnancy or neonatal
 - ◆ Drug-induced: Quinidine, heparin, sulfa compounds
 - ◆ Infections: HIV infection, infectious mononucleosis, dengue fever, cytomegalovirus
- Nonimmunological destruction
 - Disseminated intravascular coagulation
 - Thrombotic thrombocytopenic purpura, hemolytic uremic syndrome
 - Mechanical destruction: Prosthetic heart valves, malignant hypertension
 - Microangiopathic hemolytic anemias
 - Giant hemangioma

2. Decreased production of platelets

- Bone marrow failure: Aplastic anemia (congenital and acquired)
- Bone marrow replacement: Leukemia, disseminated cancer granulomatous disease
- Selective impairment of platelet production
 - Drug-induced: Alcohol, thiazides, cytotoxic drugs
 - Infections: Measles, human immunodeficiency virus (HIV)
- Ineffective hematopoiesis: Myelodysplastic syndromes
 - Nutritional deficiencies: Vitamin B₁₂, folic acid deficiency (megaloblastic anemia)

3. Sequestration

- Hypersplenism

4. Dilutional transfusions

IMMUNE THROMBOCYTOPENIC PURPURA

Q. Describe the etiology, pathogenesis, clinical features, laboratory findings/hematological features of immune/idiopathic thrombocytopenic purpura.

- Most common form of thrombocytopenia.
- **Due to increased destruction of platelets by immune mechanisms**—mainly autoimmune mechanism.

Types of Immune Thrombocytopenic Purpura (ITP)

Acute Immune Thrombocytopenic Purpura

Acute ITP is seen mainly in children between 2–4 years.

Acute ITP: Autoimmune disease, sudden onset, shorter duration and usually resolves within 6 months.

- **Self-limited** disease.
- **Children:** 2–4 years and seen equally in both sexes.
- Presents **1–3 weeks after viral** (measles, rubella, EBV) **infection**.
- Platelet destruction by **antiplatelet autoantibodies**.
- **Platelet count is decreased**, sometimes even below 10,000/cu mm ($10 \times 10^9/L$).

Clinical Features

- **Sudden onset**.
- **Petechiae, gum bleeding, epistaxis** and mild fever.
- Usually resolve spontaneously **within 6 months**.
- **Excellent prognosis**.

Chronic Immune Thrombocytopenic Purpura

- Persistent **thrombocytopenia** for **more than 6–12 months**.
- Indolent, females are more affected than males (F:M=3:1).
- **More common** and usually seen in **adults (20–40 years)**.

Chronic ITP: Autoimmune disease and the antibodies are directed against glycoprotein IIb/IIIa of platelets.

Pathogenesis of ITP (Fig. 12.1)

- **Autoimmune disorder** characterized by formation of **antiplatelet antibodies**, directed against membrane glycoproteins (most often IIb-IIIa or Ib-IX of platelets).
- Antiplatelet antibodies in about 80% of patients and are of the **IgG type**.

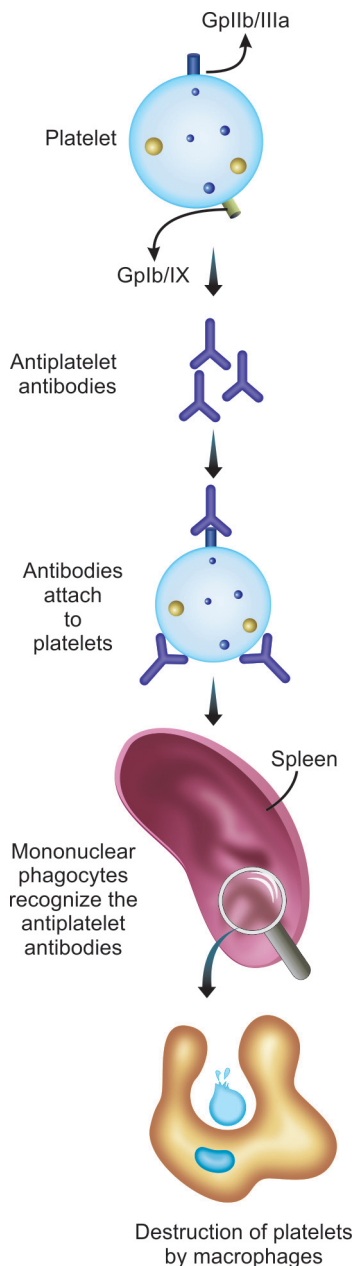


Fig. 12.1: Pathogenesis of idiopathic thrombocytopenic purpura

- Antiplatelet antibodies act as opsonins and are recognized by IgG Fc receptors present on mononuclear phagocytes of RE system (mainly spleen) and are destroyed there resulting in thrombocytopenia.
- **Splenectomy causes marked improvement** in 75–80% of patients.

Spleen is the major site of destruction of platelets and important site of autoantibody synthesis.

Clinical Features

- More common in **females** (F:M ratio is 3:1).
- Age between **20 and 40 years**.
- Clinical features are due to thrombocytopenia: **Skin bleeding, mucosal bleeding, menorrhagia** in females, etc.

ITP: Splenomegaly and lymphadenopathy are uncommon and in their presence one should consider the diagnosis other than ITP.

Laboratory Findings

Q. Write short note on laboratory findings in ITP.

Peripheral Blood

- **Platelet count:** Markedly reduced below 80,000/cu mm ($80 \times 10^9/L$).
- **Hemoglobin:** Ranges from 7–12 g/dL.
- **Peripheral smear**
 - **Platelets:** Markedly reduced (thrombocytopenia) and abnormally large sized platelets (megathrombocytes/giant platelets).
 - **RBCs:** Chronic blood loss (e.g. menorrhagia) due to ITP may lead to microcytic hypochromic anemia.
 - **WBCs:** Normal.

ITP: Platelets markedly reduced below 80,000/cu mm.

Q. Write short note on bone marrow changes in ITP.

BONE MARROW

Bone marrow in chronic ITP shows megakaryocytic hyperplasia with immature megakaryocytes.

- **Cellularity:** Hypercellular.
- **Megakaryopoiesis:**
 - **Moderate increase in number** (Fig. 12.2) of both **immature and mature** forms of megakaryocytes.
 - **Immature megakaryocytes predominate** large nonlobulated single nuclei and basophilic cytoplasm.
- **Erythropoiesis:**

ITP: Bone marrow—decreased megakaryocytes—against the diagnosis of ITP.

- **Prolonged bleeding** may cause anemia leading to **normoblastic erythroid hyperplasia**.
- Constant bleeding leads to **iron deficiency** and **micronormoblastic erythroid hyperplasia**.
- **Myelopoiesis:** Normal.
- **Storage iron:** Severe and chronic bleeding causes iron deficiency with reduced iron stores.

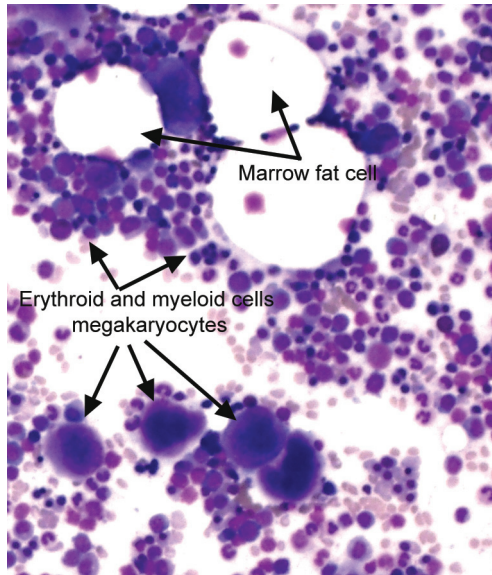


Fig. 12.2: Bone marrow in ITP showing moderate increase in number of megakaryocytes (arrows)

- **Bleeding time (BT):** Prolonged, but PT and PTT are normal.
- **Tourniquet test:** Positive.
- **Clotting time (CT):** Normal.
- **Tests for platelet autoantibodies:** May be positive.
- **Spleen:** Normal size.

ITP: Bleeding time prolonged, PT and APTT normal.

THROMBOCYTOSIS

Q. Write short note on thrombocytosis and its cause.

Platelet count more than 4,50,000/cu mm is known as thrombocytosis.

Causes: Various causes of thrombocytosis are listed in Box 12.5.

BOX 12.5: Causes of thrombocytosis

Idiopathic/primary (autonomous production)

- Essential thrombocytosis
- Polycythemia vera
- Chronic myeloid leukemia

Secondary (reactive thrombocytosis)

- Iron deficiency
- Malignancy
- Following hemorrhage
- Following splenectomy

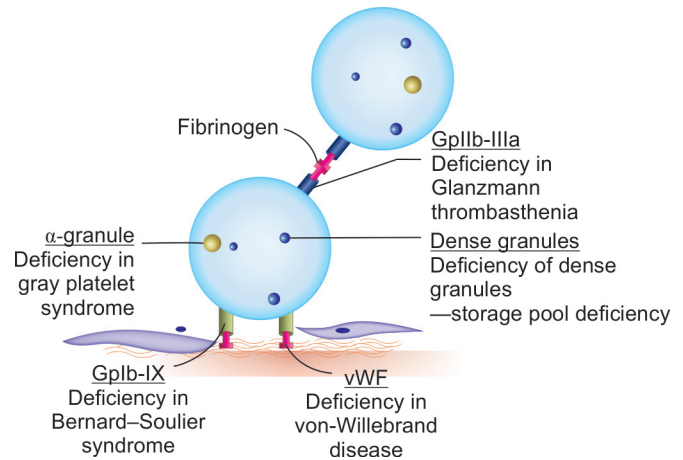


Fig. 12.3: Functional disorders of platelet

QUALITATIVE PLATELET DISORDERS

Q. Write short note on qualitative disorders of platelets.

Classification of platelet functional (qualitative) disorders are presented in Figure 12.3 and Box 12.6.

BOX 12.6: Classification of platelet functional (qualitative) disorders

A. Hereditary

1. **Disorders of platelet adhesion:** Bernard-Soulier syndrome
2. **Disorders of platelet secretion:** Storage pool deficiency
3. **Disorders of platelet aggregation:** Glanzmann thrombasthenia

B. Acquired

1. **Drugs:** Aspirin, nonsteroidal anti-inflammatory drugs (NSAIDs), dipyridamole, sulfinpyrazone
2. **Renal failure:** Uremia
3. **Hematologic malignancies:** Myeloproliferative neoplasms and myelodysplastic syndromes

Aspirin blocks the cyclo-oxygenase enzyme of platelets and prevents aggregation of platelets.

BLEEDING DISORDERS: DUE TO ABNORMALITIES OF COAGULATION/ CLOTTING FACTOR

INTRODUCTION

Bleeding due to coagulation disorders must be distinguished from those due to platelet/vascular disorders (Table 12.1).

TABLE 12.1: Distinguishing patterns of bleeding in platelet/vascular and coagulation disorders

Characteristics	Platelet/Vascular disorders	Coagulation disorders
Onset	Spontaneous and develops immediately after trauma/surgery	Delayed bleeding after trauma/ surgery
Type of lesion	Petechiae, ecchymoses	Hematomas
Sites	Skin, mucous membrane	Deep tissues
• Mucous membrane	Common from nose, mouth, gastrointestinal and genitourinary tracts	Uncommon except from gastrointestinal or genitourinary tract
• Into the joint	Absent	Common in severe factor deficiencies
• Into the muscle	Following trauma	Spontaneous

CLASSIFICATION OF COAGULATION DISORDERS (BOX 12.7)

BOX 12.7: Classification of coagulation disorders

A. Hereditary coagulation disorders

1. Hemophilia A
2. Hemophilia B
3. von Willebrand disease
4. Others

B. Acquired (secondary) coagulation disorders

1. Vitamin K deficiency
2. Liver disease
3. Others

Hereditary Coagulation Disorders

Usually, due to deficiency of single coagulation factor.

Factor VIII–vWF Complex

vWF is synthesized by endothelial cells and megakaryocytes. vWF may be located in the plasma and subendothelial tissue.

- **Factor VIII–vWF complex** has two components:
 - Plasma **factor VIII**
 - **von Willebrand factor**.
- **vWF protects factor VIII** and important for its stability. **Subendothelial vWF promotes platelet adhesion.**

- Whenever there is vascular endothelial injury, **plasma vWF gets adsorbed to exposed subendothelial matrix** and **augments adhesion of platelets**.

HEMOPHILIA

Q. Write short essay/note on hemophilia.

Three common hereditary disorders are as follows:

1. Hemophilia A (deficiency of factor VIII)
2. Hemophilia B (deficiency of factor IX)
3. von Willebrand disease (deficiency of vWF).

- Hemophilia A and B are **similar in both clinical and pathological features**, the difference being in the **deficient factor**.
- Both are **sex-linked recessive disorders** resulting in inherited deficiency of the clotting factor or synthesis of a defective clotting factor.
- **Males are affected and females are carriers.**

HEMOPHILIA A (FACTOR VIII DEFICIENCY)

Q. Write short essay/note on hemophilia A.

- **Common hereditary X-linked recessive disease.**
- About 30% of hemophiliacs may be due to acquired mutations.
- **Reduced amount or activity of factor VIII** is associated with **life-threatening bleeding**
- Bleeding is due to both inadequate coagulation and inappropriate clot removal (fibrinolysis).

Mode of Inheritance (Fig. 12.4)

Hemophilia A: X-linked recessive disorder.

- X-linked recessive disease. **Genes** for factor VIII are located **on the long arm of the X-chromosome**.
- Does not manifest when there is a normal copy of X-chromosome.
- **Males** with a defective/mutant factor VIII gene (hemophiliac gene) on their single X chromosome (X_H) **suffer from hemophilia**.
- **Heterozygous females are carriers** and do not express the full clinical disease because of the paired normal X-chromosome.
- However, females with two copies of the defective X_H chromosome may rarely suffer from hemophilia.

Hemophilia A: Males are sufferers and females are carriers.

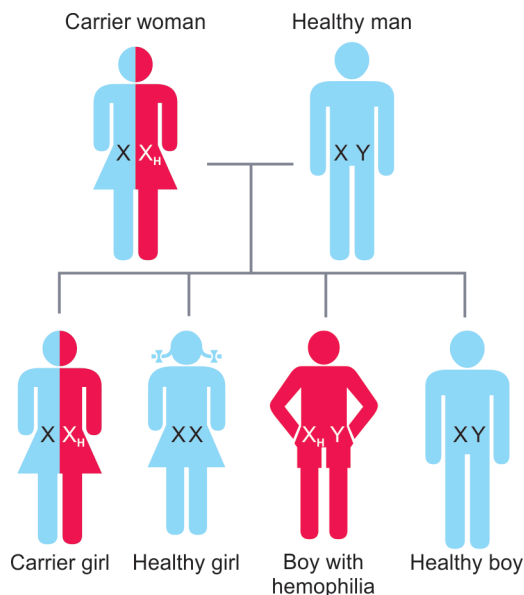


Fig. 12.4: Mode of inheritance in hemophilia

Molecular Genetics

Causative mutations include deletions, inversions, point mutations and insertions.

Clinical Features

Clinical severity depends on the level of factor VIII activity with normal range expressed as percentage (Table 12.2). Severe cases have less than 1% residual factor VIII activity.

Common clinical presentations include:

Hemophilia A: Percentage of level of factor VIII activity correlates with severity of disease.

- Frequent and spontaneous **hemorrhage into the joints**—hemarthrosis.

- Hemorrhage into **soft tissues**.
- **Prolonged bleeding** following trauma.

Hemophilia A: Common presentation

- Hemarthrosis
- Hemorrhage into soft tissues.

Laboratory Findings

- **Bleeding time:** Normal
- **Clotting time:** Prolonged, but is not a sensitive test
- **Platelet count:** Normal
- **Prothrombin time:** Normal
- **Activated partial thromboplastin time (APTT):** Increased (normal 30–40 seconds)
- **Factor VIII assay:** Essential for the diagnosis and to assess the levels and severity of disease.

Hemophilia A:

Decreased: Factor VIII

Increased: APTT and clotting time.

- **Fibrinogen assay:** Normal
- **FDP:** Negative
- **Detection of carriers:** By DNA markers
 - To detect female carriers
 - Prenatal diagnosis of affected fetuses.

Complications

Due to Hemophilia

- **Deforming arthritis and contractures:** This is due to repeated bleeding into the joints. Organization and fibrosis of intramuscular hematomas → contractures of involved muscles.
- **Anemia:** Excessive, spontaneous or repeated bleeding leads to anemia.

TABLE 12.2: Factor VIII level and clinical severity in hemophilia A

Clinical severity	Level of factor VIII activity in percentage	Clinical features
Mild	More than 6	In the mildest form, it may be unnoticed. Bleeding develops after trauma only
Moderate	2–5	Bleeding after trauma, including dental and other surgical trauma. Easy bruising
Severe	Less than 1	Frequent and spontaneous hemorrhage into joints (hemarthrosis) and soft tissues

Normal range for factor VIII: 45–158 IU/dL.

Due to Therapy

- **Viral hepatitis:** Hepatitis B, C and D in patients who received multiple transfusions of FFP/cryoprecipitate.
- **AIDS:** In individuals who received fresh frozen plasma (FFP) or cryoprecipitate, when screening tests for HIV were not available.
- **Factor VIII inhibitors:** Makes further management difficult.

Causes of death in hemophilia:

- Intracranial hemorrhage
- Prolonged bleeding.

Treatment of hemophilia:

- Factor VIII concentrate
- Recombinant factor VIII.

HEMOPHILIA B (CHRISTMAS DISEASE, FACTOR IX DEFICIENCY)

Q. Write short note on Christmas disease.

Hemophilia B:

- X-linked recessive disorder
- Mutation in factor IX
- Deficiency of factor IX.
- **Clinically indistinguishable from hemophilia A**
- **X-linked recessive disorder**
- **Variable clinical severity**
- Assay of factor IX should be done to diagnose Christmas disease (named after the first patient).

Laboratory Findings

Hemophilia B: Clinical features

- Usually milder than hemophilia A.
- Hemarthrosis is the common presentation.

Hemophilia B: Decreased factor IX and increased APTT and clotting time.

Similar to hemophilia A.

- **Bleeding time:** Normal
- **Clotting time:** Prolonged
- **Platelet count:** Normal
- **Prothrombin time:** Normal
- **Activated partial thromboplastin time (APTT): Increased** (normal 30–40 seconds)
- **Factor IX assay:** Factor IX is decreased.

VON WILLEBRAND DISEASE (VWD)

Q. Write short essay/note on von Willebrand disease, its clinical features and laboratory investigations.

vWF: Causes platelet adhesion and prevents degradation of Factor VIII in plasma. Platelet adhesion molecule is synthesized in the Weibel–Palade bodies in endothelial cells.

- Most common **inherited** bleeding disorders
- Most cases are **autosomal dominant disorders**
- Variable clinical picture with more than 20 variants.

Categories

vWD: Autosomal dominant disorders caused by mutations in vWF.

Grouped into two major categories:

- **Quantitative deficiency of vWF:** Decreased circulating vWF
 - Type 1—Autosomal dominant, mild disorder and form about 75% of all cases
 - Type 3—Autosomal recessive, severe disorder and least common type.
- **Qualitative defects in vWF:**
 - Type 2—Autosomal dominant, accounts for 25% with several subtypes.

Clinical Features

- Most cases are of **mild bleeding**
- Common symptoms
 - Spontaneous bleeding from mucous membranes (e.g. epistaxis)
 - Excessive bleeding from wounds or menorrhagia.
- In severe cases, similar to hemophilia A.

Laboratory Findings

- **Platelet count:** Normal
- **Bleeding time:** Prolonged
- **Clotting time:** Prolonged
- **Tourniquet test (Hess test):** Positive due to defect in platelet adhesion
- **APTT:** Prolonged APTT
- **PT:** Normal
- **vWF assay:** Plasma level of active vWF is decreased
- **Platelet function test:** Defective ristocetin-induced platelet aggregation test is diagnostic of vWF.

vWD: Increased bleeding time, clotting time and prolonged APTT. Plasma vWF is decreased. Defective ristocetin-induced platelet aggregation test is diagnostic.

TABLE 12.3: Summary of laboratory tests in hereditary coagulation disorders

	<i>Hemophilia A</i>	<i>Hemophilia B</i>	<i>von Willebrand disease</i>
Bleeding time	N	N	Increased
APTT	Increased	Increased	Increased
Factor VIII	Decreased	N	Low or normal
Factor IX	N	Decreased	N
vWF	N	N	Decreased

Abbreviation: N, normal

Hemophilia A, B and vWD: Prothrombin time, thrombin time and platelet count are normal. APTT increased in all the three.

Laboratory tests in hereditary disorders are summarized in Table 12.3.

ACQUIRED COAGULATION DISORDERS

Vitamin K dependent coagulation factors: II, VII, IX and X.

Q. Write short note on acquired coagulation disorders and its causes.

Coagulation Factor Abnormalities

Usually characterized by multiple clotting abnormalities

- **Vitamin K deficiency:** In neonates, low levels of vitamin K levels may produce life-threatening hemorrhage during the first week of life known as *hemorrhagic disease of the newborn*.
- **Liver disease:** Liver synthesizes all the clotting factors and severe liver disease is associated with a hemorrhagic diathesis.
- **Other causes:** Disseminated intravascular coagulation that involves deficiency of several coagulation factors.

DISSEMINATED INTRAVASCULAR COAGULATION

Q. Write short essay/note on disseminated intravascular coagulation, etiology, pathogenesis, clinical features and laboratory investigations.

Widespread disorder with **combination of thrombosis and hemorrhage**.

Etiology

Develops as a secondary complication of wide variety of disorders (Box 12.8).

BOX 12.8: Major disorders associated with disseminated intravascular coagulation

Infections

- Gram-negative bacterial sepsis
- Meningococemia and other bacteria
- Fungi, viruses, Rocky Mountain spotted fever, malaria

Obstetric Complications

- Retained dead fetus
- Abruptio placentae
- Toxemia and pre-eclampsia
- Septic abortion
- Amniotic fluid embolism

Neoplasms

- Carcinomas of pancreas, prostate, lung and stomach
- Acute promyelocytic leukemia

Massive Tissue Injury

- Traumatic
- Fat embolism
- Burns
- Surgery

Vascular Disorders

- Aortic aneurysm, giant hemangioma

Immunologic Reactions

- Transfusion reactions
- Transplant rejection

Respiratory Distress Syndrome

Miscellaneous

- Snakebite, liver disease, acute intravascular hemolysis, shock, heat stroke, hypersensitivity, vasculitis

DIC: Sepsis, major trauma, obstetric complications and certain cancers are the common triggers.

Pathogenesis (Fig. 12.5)

Disseminated intravascular coagulation (DIC) is a disorder that shows **combination of (i) thrombosis and (ii) hemorrhage**.

DIC: Widespread thrombo-hemorrhagic disorder secondary to wide variety of disorders.

Thrombi/Clot Formation

Mechanism of Thrombi Formation

- **Initiation of thrombotic process:** Two major mechanisms initiate the thrombotic process of DIC namely entry of **thromboplastic (procoagulant) substances** into the circulation and widespread **endothelial injury**.
 - *Entry of thromboplastic (procoagulant) substances into the circulation:* Source of thromboplastic/procoagulant substance in majority is **tissue factor**, which **activates** coagulation system.
 - *Widespread endothelial injury:* Endothelial injuries **expose the thrombogenic subendothelial matrix**.

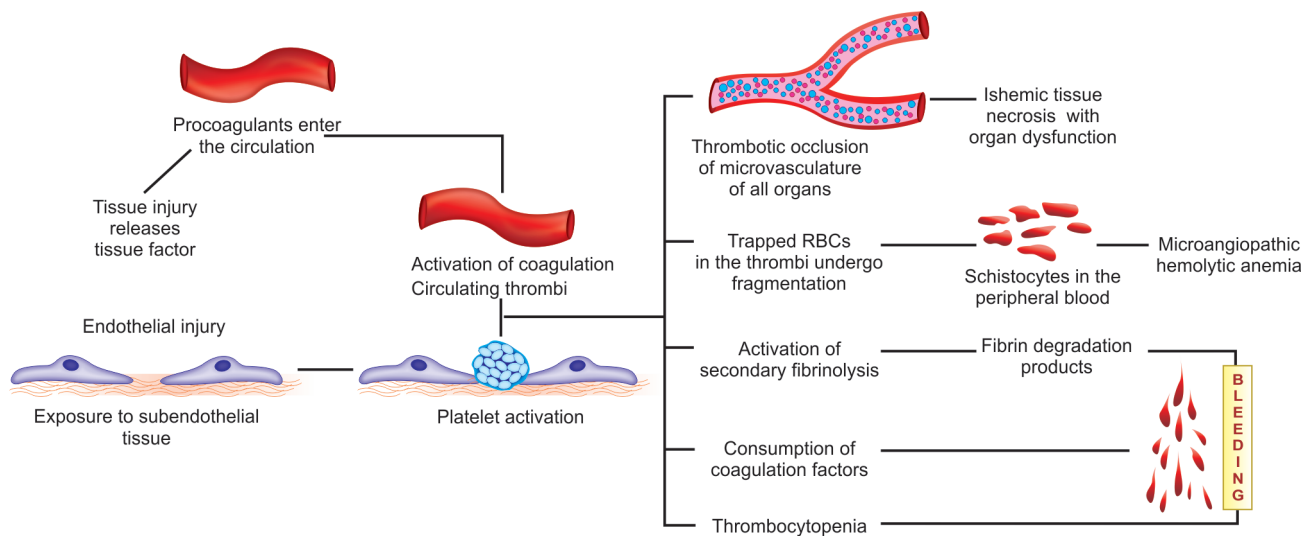


Fig. 12.5: Pathogenesis of thrombosis, ischemic tissue necrosis and bleeding in disseminated intravascular coagulation

• Development of thrombi:

- Both procoagulant substances (tissue factor) and endothelial injury activate coagulation system resulting in fibrin-platelet **thrombi formation** in the microvasculature.
- During this process there is consumption of clotting factors, fibrin and platelets. Hence, it is also referred to as **consumptive coagulopathy** or **defibrination syndrome**.

• Consequences of thrombi formation: Widespread deposition of fibrin-thrombi within the microcirculation leads to:

- Ischemic necrosis:** Microvascular thrombi produces **micro-infarcts or large areas of infarction** and multiorgan failure.
- Microangiopathic hemolytic anemia:** RBCs trapped in the intravascular fibrin-thrombi deposits undergo fragmentation. These RBCs appear as schistocytes in blood smears; but, frank hemolytic anemia is unusual in DIC.

Hemorrhagic Diathesis

- Causes of hemorrhagic/bleeding diathesis:
 - Consumption of platelets
 - Consumption of coagulation factors
 - Activation of fibrinolytic system.
- Mechanism of hemorrhagic diathesis:** Fibrin-thrombi activate secondary fibrinolytic system and generate **plasmin**. The plasmin cleaves fibrinogen and fibrin and generates fibrin split products (FSPs) [or fibrin degradation products (FDP)].

FSPs are potent anticoagulant and antiplatelet effect and produces hemorrhagic diathesis.

DIC:

- Consumption of coagulation factors
- Widespread thrombosis in small blood vessels.

Clinical Features

- Serious, often fatal, clinical condition**
- Signs and symptoms are related to:
 - Hemorrhagic diathesis/bleeding:** Most common, manifest as ecchymoses, petechiae or bleeding from mucous membranes or at the sites of venipuncture.
 - Microvascular thrombi:** Tissue hypoxia and infarction of the organ leading to multiorgan failure.

Laboratory Findings in DIC

Screening Assays

- Coagulation abnormalities**
 - APTT:** Increased as a result of consumption and inhibition of the function of clotting factors.
 - Prothrombin time:** Increased.
 - Thrombin time (TT):** Increased because of decreased fibrinogen.
 - Fibrinogen:** Decreased.
- Bleeding time:** Increased due to decreased platelet count.

- **Platelet count:** Decreased due to utilization of platelets in microthrombi.
- **Peripheral smear:** Microangiopathic hemolytic anemia with schistocytes.

DIC laboratory findings

- Increased: APTT, PT, BT, D-dimer
- Decreased: Platelets, fibrinogen.

Confirmatory Tests

- **Fibrinolysis abnormalities**
- Fibrin degradation/split products (FDP): Secondary fibrinolysis results in **generation of FDPs**, which can be measured by latex agglutination
- **D-dimer test:** It is specific for diagnosing DIC.

DIC: D-dimer test is specific diagnostic test.

Prognosis

- Depends on the underlying disorder.
- Mortality is high in severe cases.

Treatment

- Removal of the underlying cause
- Replacement of clotting factors and platelets.

Summary of screening tests for bleeding disorders are presented in Table 12.4.

THROMBOTIC DISORDERS: HYPERCOAGULABLE STATE

HYPERCOAGULABLE STATE (THROMBOPHILIA)

Group of inherited or acquired conditions that are associated with **increased tendency or risk to develop thrombosis**.

Refer Box 5.1 for Causes of Hypercoagulability

Inherited Hypercoagulable States

Clinical Presentation

- **Thrombosis** develops at **young age** (less than 45 years)
- **Recurrent** thromboembolism
- **Family history** of thromboembolic episodes
- Thrombosis develops in the venous system and at **unusual anatomical sites** like visceral veins.

Deficiency of Antithrombotic Factors

Antithrombin (AT) III Deficiency

- Autosomal dominant disorder
- Deficiency of antithrombin—either quantitative or qualitative
- Risk of a thrombosis—20–80%.

TABLE 12.4: Summary of screening tests for bleeding disorders

Investigation	Normal range	Main causes of abnormal test
Blood count and film	Show the number and morphology of platelets and any blood disorder	Platelet disorders, leukemia or lymphoma
Platelet count	150–450 × 10 ⁹ /L	Thrombocytopenia
Bleeding time (template method)	2–9 minutes	<ul style="list-style-type: none"> • Thrombocytopenia • Abnormal platelet function • Deficiency of von Willebrand factor • Vascular abnormalities
Prothrombin time (PT)	11–16 seconds	Deficiency or inhibitors of factors II, (prothrombin) V, VII or X or fibrinogen (I). Others include heparin, warfarin
Activated plasma thromboplastin time (APTT)	30–40 seconds	<ul style="list-style-type: none"> • Deficiency or inhibitors of prekallikrein; high molecular weight kininogen; factors II, V, VIII, IX, X, XI, XII or fibrinogen (I). Others include heparin, warfarin • Heparin • Antibodies against clotting factors • Lupus anticoagulant
Thrombin time	15–19 seconds	Hypofibrinogenemia, DIC

Protein C and S Deficiency

- Normally, activated **proteins C (APC) and protein S** act as a complex, which **degrades activated factors V and VIII**.
- When there is deficiency of these proteins, the activated factor V and VIII are **not neutralized**. This leads to activation of the clotting system and formation of thrombus.

Increased Prothrombotic Factors

Activated Protein C (APC) Resistance (Factor V Leiden)

Factor V Leiden/Leiden mutation is characterized by factor V variant.

Factor V Leiden is resistant to inhibition by activated protein C (APC). It is associated with familial thrombophilia.

- Most common genetic disorder associated with familial thrombophilia.
- Activated **proteins C (APC) and protein S** complex inhibits activated factor normal V and VIII. The variant clotting factors cannot be degraded.
- **Point mutation** in the **factor V gene** synthesis of a **factor V variant**. This variant is known as factor V Leiden/Leiden mutation.
- **Factor V variant** has normal procoagulant activity but is **resistant to inhibition by activated protein C (APC)**.

ACQUIRED HYPERCOAGULABLE STATES

Causes of the acquired hypercoagulable states (refer Box 5.1).

Antiphospholipid Antibody Syndrome (APLA/APS)

- Presence of antiphospholipid antibodies (APAs) in the plasma are associated with **hypercoagulable state**.
- **Antiphospholipid antibody** reacts with plasma proteins, which are bound to phospholipids (refer Fig. 6.17).
- **Two important antiphospholipid antibodies: Lupus anticoagulant antibody and anti- β_2 glycoprotein antibody.**
 1. **Lupus anticoagulant antibody: Prolongs the phospholipid-dependent coagulation tests in vitro** (e.g. prolongation of APTT).

2. **Antibodies against the phospholipid- β_2 -glycoprotein complex:** It also bind to cardiolipin antigen used in the serological test for syphilis.

Antiphospholipid antibodies includes lupus anticoagulant antibody and anti- β_2 glycoprotein antibody.

Types

- **Primary antiphospholipid syndrome:** No predisposing cause.
- **Secondary antiphospholipid syndrome:** Association with autoimmune diseases, like systemic lupus erythematosus, hence known as lupus anticoagulant syndrome.

Clinical Features

Triad of thrombosis, recurrent spontaneous abortions and immune thrombocytopenia may be the presenting clinical features of antiphospholipid syndrome.

- **Hypercoagulable state:** Commonest acquired hematologic cause of recurrent thromboembolic events.
- **Repeated spontaneous abortions:** Normally, tissue plasminogen activator (t-PA) is necessary for the invasion of uterine blood vessels by placental trophoblastic tissue. Recurrent spontaneous abortions develop due to antibody-mediated inhibition of t-PA activity.
- **Immune thrombocytopenia.**

Laboratory Tests

Coagulation Tests

- **APTT: Prolonged**
- Factor VIII levels: Normal
- Prothrombin time: Normal
- Thrombin time: Normal
- Fibrinogen level: Normal.

Confirmatory Test

- **Test for lupus anticoagulant:**
 - **Dilute Russell's viper venom test (DRVVT):** Russell's viper venom (RVV) activates factor X leading to fibrin clot. **Lupus anticoagulant prolongs clotting time** by binding to RVV and preventing the action of RVV.
- **Antibodies against the phospholipid- β_2 -glycoprotein complex:**
 - Detected by enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay (RIA).

Clinical Pathology

ANTICOAGULANTS

Q. Write short essay/note on anticoagulants/various types of anticoagulants used in hematology. Mention their mode of action. List the uses of trisodium citrate.

Blood coagulates when withdrawn from the vessel and anticoagulants are used to prevent blood from clotting. Commonly used anticoagulants are listed in Table 13.1.

TABLE 13.1: Commonly used anticoagulants

I. Calcium chelating agents	Uses
<ul style="list-style-type: none"> Ethylene diamine tetra acetic acid (EDTA) 	RBC count, total leukocyte count, eosinophil count, platelet count. Hemoglobin, HbF estimation and Hb electrophoresis
<ul style="list-style-type: none"> Double oxalate (mixtures of ammonium oxalate and potassium oxalate) 	Since, morphology of the blood cells is not well preserved, it is now replaced by EDTA
<ul style="list-style-type: none"> Sodium citrate solution 	Coagulation studies, ESR by Westergren method, blood bank {ACD (acid citrate dextrose) solution was used and now replaced by citrate phosphate dextrose adenine (CPDA)}
<ul style="list-style-type: none"> Sodium fluoride 	Estimation of blood sugar
II. Heparin	Osmotic fragility test, red cell enzyme studies (e.g. G6PD and PK deficiency), electrolyte estimation, arterial blood gas analysis

Double Oxalate

Q. Write short note on double oxalate mixture.

Potassium oxalate shrinks RBCs and **ammonium oxalate** causes swelling of RBCs. To balance the swelling effect of ammonium oxalate and the shrinking effect of potassium oxalate, the two are combined in a mixture in the ratio of three parts of ammonium oxalate to two parts of potassium oxalate. Morphology of the blood cells is not well-preserved; hence, it has now been replaced by EDTA.

Type of Blood Sample

Q. Write short note on method of obtaining plasma and serum.

- 1. Whole blood:** Is used for complete hemogram (hemoglobin, ESR, platelet count, reticulocyte count, and peripheral smear evaluation), osmotic fragility test, estimation of HbF, Hb electrophoresis, Coombs test and for biochemical investigations like blood glucose.
- 2. Serum:** Is obtained by **allowing the blood to clot** in a tube or in a vial (without adding any anticoagulant). It is used for estimation of bilirubin, creatinine, uric acid, proteins, albumin, globulin and A/G (albumin/globulin) ratio, serum enzyme levels [e.g. alkaline phosphatase, acid phosphatase, aspartate (AST/SGOT) and alanine aminotransferase (ALT/SGPT)], serum electrophoresis of proteins, lipoproteins and immunoglobulins.
- 3. Plasma:** Is obtained by **centrifugation of the anticoagulated blood**. Red blood cells form the sediment and the supernatant is the plasma. It is used for coagulation studies [prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time], assay of various coagulation factors (e.g. factor VIII, IX), assay of FDP and D-dimer (in disseminated intravascular

coagulation), confirmation of hemoglobinemia where plasma is colored red (e.g. PNH).

Collection of Blood

Venous blood for the tests is collected by (i) syringe or (ii) vacuum tubes.

Q. Write short note on vacutainer.

- **Vacutainer:** These are vacuum tubes which have a colored top (Table 13.2). They are used in most of the laboratories and replaced the collection by syringe. It is very essential to use appropriate vacuum tubes with proper ratio of anticoagulant and the blood for specific tests.

Capillary blood: May be collected either from the finger or heel pricks using sterile disposable lancet/needle.

Arterial blood: Is usually required for blood gas analysis and is collected from the femoral artery by disposable needle and syringe.

Complications Encountered During Blood Collection

Q. Write short note on complications of venipuncture.

- Ecchymosis and hematoma at the site of puncture.
- Syncope or fainting.
- Failure to get blood because the needle might not have entered the vessel. In obese individuals, it is often difficult to locate the vein.
- Hemolysis of blood sample due to narrow bore of needle, quick withdrawal of blood with excessive suction or contamination of syringe by water. Hemolyzed blood sample is not suitable for testing.

TABLE 13.2: Types of vacuum tubes, its color, content and uses

Color of the top	Anticoagulant	Uses
Red	No chemicals—plain	Serum for various studies
Purple or lavender	EDTA	Routine hematological tests
Light blue	Sodium citrate	Coagulation studies
Gray	Fluoride	For glucose estimation
Green	Heparin	Osmotic fragility test, red cell enzyme study
Black	Citrate	Erythrocyte sedimentation rate

HEMOGLOBIN ESTIMATION

Q. Write short note on methods of hemoglobin estimation.

One of the important features of anemia is reduction in the red cell mass. Red cell mass can be estimated by measuring hemoglobin (Hb). There are several methods for Hb estimation utilizing different principles (Table 13.3). The commonly used methods include colorimetric method namely Sahli's and cyanmethemoglobin method.

TABLE 13.3: Methods of hemoglobin estimation

Method	Principle
Colorimetric method	
1. Visual colorimetric method; Sahli's method or acid hematin method, alkaline hematin method	These methods are based on measuring the color of hemoglobin or a hemoglobin derivative in the blood either visually or by photolorimetry
3. Photoelectric method: Cyanmethemoglobin (HiCN) method, oxyhemoglobin method	
3. Haldane method	
Physical (specific gravity) method	Specific gravity
Chemical method	Iron content of hemoglobin
Gasometric method	Oxygen combining capacity of hemoglobin
Cell counter/autoanalyzer	RBCs are lysed by the lysate and color is matched against inbuilt standard color. Most use cyanide-free biodegradable reagent

COMPLETE BLOOD COUNTS (HEMOGRAM) (TABLE 13.4)

Q. Write short note on complete blood counts (CBC) (hemogram).

TABLE 13.4: Complete blood counts (CBC)

Hemoglobin	Reticulocyte count
Total leukocyte count	Peripheral blood smear
RBC count	• RBC morphology
Platelet count	• Platelets morphology
Differential leukocyte count	• Hemoparasites
Hematocrit (Hct)/packed cell volume (PCV)	• Any abnormal/atypical cell

Various Parameters Obtained In Automated Cell Counters (Table 13.5)

Q. Write short note on various parameters obtained in automated cell counters.

TABLE 13.5: Various parameters obtained in automated cell counters

Hemoglobin	Mean platelet volume (MPV)
Absolute values:	Hematocrit (Hct)/ packed cell volume (PCV)
• Mean corpuscular volume (MCV)	Total leukocyte count
• Mean corpuscular hemoglobin (MCH)	RBC count
• Mean corpuscular hemoglobin concentration (MCHC)	Platelet count
• Red cell distribution width (RDW)	Reticulocyte count
	Differential leukocyte count (3 part or 5 part)

PERIPHERAL BLOOD SMEAR EXAMINATION

Importance of Peripheral Smear Examination

Q. Write short essay on importance of peripheral smear examination.

Peripheral smear (peripheral blood film) is the most important, valuable and frequently asked investigation in hematology laboratory. It provides the following information:

- **Red cell morphology:** Morphological features (size, shape) of RBCs are important for diagnosis of anemias and other hematologic disorders (Figs 13.1 to 13.4).
- **WBC disorders and differential leukocyte count (DLC):** Quantitative and qualitative changes in WBCs help in diagnosis of both hematologic and nonhematologic disorders.
- **Platelet number and morphology:** These features are useful in the diagnosis of bleeding disorders.
- **Cross check the CBC parameters:** It helps in cross-checking the complete blood count (CBC) parameters derived from automated cell counters.
- Detection of **blood parasites** (hemoparasites).

Q. Write short note on poikilocytosis, anisocytosis, macrocyte and target cell.

Stains for Blood Smear

Blood cells contain cellular structures which vary in their reaction (pH), some are acidic and others being basic. The aniline dyes used in staining blood smears are of two general classes: Basic dyes, such as methylene blue and acidic dyes, such as eosin.

Romanowsky Stains

Q. Write short essay on Romanowsky stains.

All stains which are made of combinations of acidic (eosin) and basic dyes (methylene blue) are called **Romanowsky stains**. The action of these stains depends on compounds



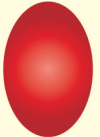
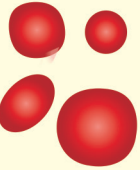
Term	Description	Appearance	Condition
Normocyte	• Normal RBC is biconcave, 7.2 μm in diameter		• Normal RBC • Normocytic normochromic anemia
Microcyte	• RBCs smaller than normal and less than 6 μm in diameter		• Iron deficiency anemia • Thalassemia
Macro-ovalocyte	• Larger than normal, more than 9 μm in diameter and also oval		• Megaloblastic anemia due to vitamin B ₁₂ and folic acid deficiency
Anisocytosis	• Variation in size		• Severe anemia of any type

Fig. 13.1: Variation in size of red blood cells and associated conditions














Term	Description	Appearance	Condition
Normal	• Biconcave, flattened on smear		• Normal
Poikilocytosis	• Variation in shape		• Severe anemia
Spherocytes	• Smaller and stain darker without any central pallor		• Hereditary spherocytosis • Autoimmune hemolytic anemia
Target cells or leptocytes	• Only the periphery and the central regions of the cell appear hemoglobinized		• Thalassemia • Sickle cell anemia • Hemolytic anemia • Post-splenectomy • Liver disease
Sickle cells	• Thin, elongated, slightly curved and have shape of a sickle		• Sickle cell anemia
Bite cells	• Pairs of spicules		• G6PD deficiency
Schistocytes (fragmented cells)	• Small, irregular shaped, triangular or speculated cells		• Microangiopathic hemolytic anemia (e.g. DIC, TTP)
Acanthocytes	• RBCs with few spicules of uneven (irregular) length and shape		• Abetalipoproteinemia • Liver disease
Echinocyte/ Burr cell	• Very small irregular shrunken cells with pointed projections. Resemblance to the small thorny 'burrs'		• Uremia
Elliptocyte or ovalocyte	• Oval in shape		• Hereditary ovalocytosis • Hemolytic anemia
Pencil-shaped cells	• Elongated thin cells (exaggerated ovalocytes)		• Iron deficiency anemia
Teardrop and pear-shaped cells	• These abnormal RBCs have a teardrop or pear-like shape		• Myelofibrosis • Marrow infiltration
Stomatocytes	• Red cells with a slit-like area of central pallor		• Hereditary stomatocytosis • Liver disease

Fig. 13.2: Variation in shape of red blood cells and associated conditions

formed by the interaction of methylene blue and eosin. Methylene blue on oxidation produces colored compounds called azures that have the ability to combine with eosin.

Oxidation is achieved during maturation/chemical treatment of the stain. The azures are responsible for different shades of staining in the smears (i.e. RBCs— pink, granules of




Term	Description	Appearance	Condition
Normochromic	<ul style="list-style-type: none"> Cell with central 1/3 pallor 		<ul style="list-style-type: none"> Normal RBC
Hypochromic	<ul style="list-style-type: none"> Central pallor more than 1/3 		<ul style="list-style-type: none"> Iron deficiency anemia Thalassemia Anemia of chronic disorder
Polychromasia	<ul style="list-style-type: none"> Cells stained purple and are larger than normal 		<ul style="list-style-type: none"> Hemolytic anemia Response to therapy in deficiency anemia

Fig. 13.3: Variation in color of red blood cells

eosinophils—red-orange, granules of basophils—bluish-black, granules of neutrophils—lilac).

- Nuclei and structures in the blood which are stained by the basic dyes are called basophilic.
- Structures that take up only acidic dyes are called acidophilic or eosinophilic.

Romanowsky group includes the following stains:

- **Leishman stain** contains acetone-free methyl alcohol which acts as a **fixative**. Since, acetone destroys the cells, methyl alcohol should be free from acetone.
- **Giemsa stain**
- **Wright stain**
- **Jenner stain**
- **Jenner–Giemsa stain**

The differences between the various Romanowsky stains are mainly in the proportion of the reagents and in their preparation.

Hemoparasites

Q. Write short essay on hemoparasites.

Hemoparasites include (i) malarial parasites, (ii) microfilaria, (iii) trypanosomes, (iv) *Leishmania donovani* and (v) babesiosis.

1. **Malaria:** Malarial parasites can be demonstrated in the peripheral smear; most common are *Plasmodium vivax* and *plasmodium falciparum*.
 - **Plasmodium vivax:** In the peripheral blood the trophozoites, schizonts and rarely gametocytes of plasmodium vivax may be found.

- **Plasmodium falciparum:** In falciparum infections usually only ring stage and gametocytes may be demonstrated.

2. **Filaria:** *Microfilaria* can also be demonstrated in peripheral blood.
3. **Trypanosomes:** These are motile flagellate protozoa.
4. **Leishmania donovani (LD):** It causes **kala-azar**. Amastigote forms known as LD bodies can be found in the reticuloendothelial cells of the bone marrow, spleen and buffy coat preparations of peripheral blood.
5. **Babesiosis:** It is a malaria-like parasitic disease caused by babesia.

RETICULOCYTE COUNT

Q. Write short essay on reticulocyte, its morphology, staining method, normal values and its importance. Note on conditions in which reticulocyte count is increased.

Reticulocytes are **immature, non-nucleated RBCs** released from bone marrow. They are slightly larger than the mature RBCs. They continue to synthesize hemoglobin after loss of the nucleus.

Methods of Reticulocyte Count

- **Visual method:** Staining in living state is known as **supravital staining**. Reticulocytes contain ribosomes and RNA and can be stained by supravital stains in the live and unfixed state. When blood is briefly incubated in supravital stains, such as **new methylene blue** or

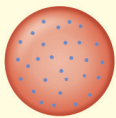
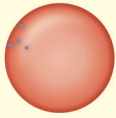
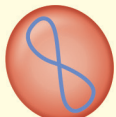
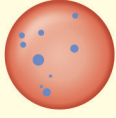

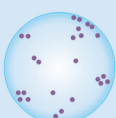
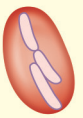
Term	Description	Appearance	Condition
Basophilic stippling (punctate basophilia)	<ul style="list-style-type: none"> Red cells contain small, blue black inclusions and represent precipitated ribosomal RNA 		<ul style="list-style-type: none"> Lead poisoning Megaloblastic anemias Hemolytic anemias like thalassemias Sideroblastic anemias Alcoholism
Howell-Jolly bodies	<ul style="list-style-type: none"> Small, round densely staining dark blue particles and represent nuclear remnants 		<ul style="list-style-type: none"> Megaloblastic anemias Acute hemolytic anemias Post splenectomy state
Cabot rings	<ul style="list-style-type: none"> Rings or 'figure of eight' shaped and are probably remnants of the nuclear membrane 		<ul style="list-style-type: none"> Hemolytic anemia Megaloblastic anemia Leukemia Rarely after splenectomy
Pappenheimer bodies/siderotic granules (RBCs with Pappenheimer bodies are called siderocytes)	<ul style="list-style-type: none"> Aggregates of ferritin and appear pale blue, but are easily demonstrable with Perl's stain (Prussian blue reaction) 		<ul style="list-style-type: none"> Sideroblastic anemias Megaloblastic anemias Hemolytic anemias Post splenectomy
Hemoglobin H inclusions	<ul style="list-style-type: none"> Free β chains in red cells. Small, regular, multiple and diffusely distributed (golf ball like appearance). Stained by supravital stains. 		<ul style="list-style-type: none"> α-thalassemias
Heinz bodies	<ul style="list-style-type: none"> Refractive, single or multiple rounded inclusions of denatured globin detected by supravital stains 		<ul style="list-style-type: none"> Intake/exposure to oxidizing drugs or chemicals G6PD deficiency Unstable hemoglobin disease
Hemoglobin C crystals	<ul style="list-style-type: none"> Crystallization of hemoglobin C 		<ul style="list-style-type: none"> Splenectomy in homozygous hemoglobin C

Fig. 13.4: Inclusions in red blood cells and associated conditions

brilliant cresyl blue solution, the RNA is precipitated as a dye-ribonucleoprotein complex (refer Fig 10.14). On microscopy, the complex appears as a dark blue network (reticulum or filamentous strand or granular material). Reticulocytes stain *polychromatic* with Romanowsky stains and hence, the term "*polychromatophil*" is used to indicate their presence in peripheral smear.

- Automated method.

Normal reticulocyte count: Reticulocyte count is expressed as percentage of total red cells. Normal is 0.5–2.5% and in newborn (cord blood) it is 1–7.0%.

Causes of increased and reduced reticulocyte count are listed in Table 13.6.

HEMATOCRIT (Hct)

Q. Write short essay/note on definition, methods and significance of PCV/hematocrit.

Q. Write short essay/note on Wintrobe PCV tube and its uses.

Hematocrit (packed cell volume/erythrocyte volume fraction) is the ratio of the volume of red cells to that

TABLE 13.6: Causes of increased and reduced reticulocyte count

<i>Causes of increased reticulocyte count</i>	<i>Causes of reduced reticulocyte count</i>
<ul style="list-style-type: none"> • Hemolytic anemias • Hemolytic crisis • Hemorrhage • Following treatment in iron/folic acid/vitamin B₁₂ deficiency anemias. Highest counts are found on 6th/7th day of treatment and indicate marrow response to hematinics 	<ul style="list-style-type: none"> • Due to decreased erythropoietic activity • Aplastic anemia • Aplastic crisis due to parvovirus (hereditary spherocytosis and sickle cell disease) • Pure red cell aplasia • Fanconi anemia • Myelofibrosis

of the whole blood. It indicates relative volume of red cells and plasma (e.g. in anemia red cells are reduced with corresponding reduction in the hematocrit).

Methods of Estimation of PCV

- Macromethod using Wintrobe tube.
- Micromethod using capillary tube.
- Automated analyser.

Wintrobe Method

Wintrobe tube (Fig. 13.5): It is a special thick-walled glass tube measuring **11 cm in length** and an internal diameter of 2.5 mm, with a capacity of 1 mL. This is calibrated at 1 mm intervals up to 105–110 mm. It has bold markings in ascending order from top as 0, 10, 20, 30,...100 for ESR determination and descending order from the top as 100, 90, 80,...10, 0 for PCV estimation.

Anticoagulants used: EDTA, dried heparin or double oxalate.

Principle: Anticoagulated whole blood is centrifuged at a standard speed. RBCs which are heavier than white cells, platelets and plasma, sediment at the bottom and the volume of red cell mass denotes the hematocrit.

Different layers (Fig. 13.5)

- **Lower layer:** This consists of red blood cells (packed).
- **Middle layer:** It is also called **buffy coat** and is the thin red-gray layer between the red cells and plasma consisting of WBC and platelets.
- **Upper layer:** This layer is composed of the plasma which is normally clear or straw colored.

Normal range for PCV: Hematocrit is expressed as a percentage (e.g. 45%) or as a decimal fraction (e.g. 0.45). It is useful for evaluating absolute values like MCV and MCHC.

- Adult males 38–47%
- Adult females 36–46%
- Infants 45–70% (cord blood)

Causes of Increased PCV: Polycythemia vera rubra and secondary polycythemia. **Decreased PCV** is seen in anemia.

Uses of Wintrobe Tube and Hematocrit

- **For estimation of hematocrit** which is useful for the following:
 - **Anemia:** To determine the presence of anemia, to assess its severity and to assess response to therapy.
 - Presence or absence of **polycythemia**.
 - **Checking the accuracy of hemoglobin value** (Hb in g/dL $\times 3$ = hematocrit).

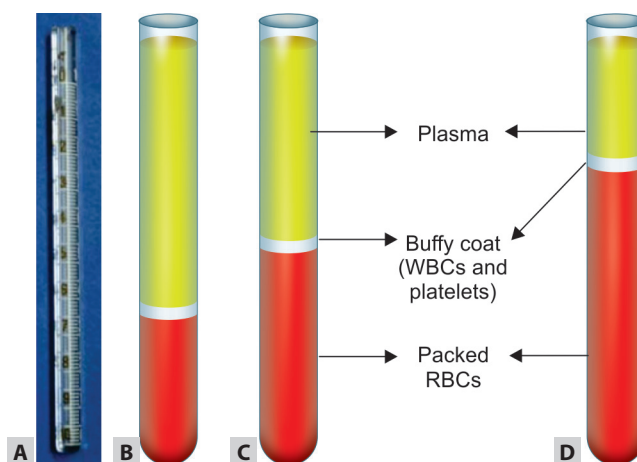


Fig. 13.5: (A) Wintrobe tube and diagrammatic appearance of different columns after the blood is centrifuged in (B) anemia; (C) normal; and (D) polycythemia vera

- PCV which is also required to **determine the red cell indices** namely mean cell volume (MCV) and mean cell hemoglobin concentration (MCHC).
- **Buffy coat obtained by hematocrit** gives an approximate indication of the number of WBCs (normally 0.1 mm of this layer = 1000 WBC/cu mm). Uses of buffy coat are as follows:
 - Thickness of the **buffy coat increases with marked leukocytosis**, as in CML, CLL and acute leukemias. Absent or minimal buffy coat implies leukopenia.
 - In subleukemic leukemia, a smear made from the buffy coat has greater concentration of WBCs and identification of abnormal cells is easier.
 - LE cell test.
- **ESR estimation:** However, the Westergren method is preferred as it is more accurate.

ERYTHROCYTE SEDIMENTATION RATE

Q. Write short essay/note on normal values, indications, anticoagulants used, stages and factors affecting erythrocyte sedimentation rate.

Erythrocyte sedimentation rate (ESR) estimation is a commonly used nonspecific test in routine clinical practice. ESR is a useful but nonspecific marker of underlying inflammation. However, other high-sensitivity inflammatory markers (e.g. C-reactive protein) are presently used to detect or monitor disease (e.g. cardiovascular disease and metabolic syndrome).

Principle: When anticoagulated blood is placed in a vertical tube and is allowed to stand, RBCs settle towards the bottom of the tube. The speed of sedimentation of red cells in plasma over a period of 1 hour is measured by the length of the sedimented RBC column and is expressed in millimeters. RBCs have net negative charge on their surface and tend to repel each other. The repulsive forces are partially or totally counteracted if there is an increase in the positively charged plasma proteins.

Factors Affecting ESR (Box 13.1)

- **Plasma factors:** An accelerated ESR is favored by elevated levels of fibrinogen, globulins and cholesterol (which increase the positive charge of plasma) whereas albumin and lecithin retard ESR.
- **Red cell factors:** The sedimentation rate is directly proportional to the weight of the cell aggregates and inversely proportional to the surface area.

BOX 13.1: Factors that affect ESR

Factors that increase ESR: Old age, pregnancy, anemia, macrocytosis, raised fibrinogen

Factors that decrease ESR: Microcytosis, polycythemia, marked leukocytosis, low fibrinogen

- **Number:** Anemia increases the ESR and polycythemia decreases.
- **Size:** Microcytes sediment slower than macrocytes.
- **Rouleaux formation:** It accelerates the ESR.
- Red cells with an **abnormal or irregular shape**, such as sickle cells or spherocytes, do not exhibit rouleaux formation and have low ESR.
- **Technical factors:** ESR tube must be kept vertical; otherwise it results in inaccurate ESR.

Stages of ESR

Sedimentation occurs in three stages:

1. **Stage of aggregation/rouleaux formation:** In the initial 10 minutes, there is little sedimentation as rouleaux form and the size of the rouleaux formed influence the speed of sedimentation.
2. **Stage of settling:** For about 40 minutes, settling occurs at a constant rate.
3. **Stage of packing:** Packing of RBCs occur in the final 10 minutes.

Methods

Two commonly employed methods are as follows: **Westergren and Wintrobe methods.**

Westergren Method

Q. Write short note on Westergren pipette/tube.

Westergren tube is a straight glass pipette (open at both ends) 30 cm in length, bore of 2.55 mm and calibrated in millimeters from 0–200. The capacity of tube is about 1 mL. The tube is vertically placed on the Westergren rack.

Anticoagulant used: 3.8% trisodium citrate.

Ratio of blood and anticoagulant: 2 mL of whole blood in 0.5 mL of 3.8% trisodium citrate. Blood to anticoagulant ratio should be 4:1.

Indications for ESR estimation (Box 13.2).

Normal range for erythrocyte sedimentation rate (Table 13.7).

BOX 13.2: Indications for ESR

- Suspicion of infectious, inflammatory or neoplastic conditions
- To monitor disease activity: For example, rheumatic fever, tuberculosis
- As a diagnostic criteria: For example, temporal arteritis

TABLE 13.7: Revised normal range for erythrocyte sedimentation rate

Age	Men	Women
Below 50 years	15 mm/hour	20 mm/hour
Above 50 years	20 mm/hour	30 mm/hour
Above 85 years	30 mm/hour	42 mm/hour

TABLE 13.8: Causes of increased ESR**Markedly increased in:**

- Multiple myeloma
- Macroglobulinemia
- Hyperfibrinogenemia

Moderately increased in:

- Infective diseases like tuberculosis
- Chronic inflammatory diseases
 - Rheumatic fever, osteomyelitis
 - Autoimmune diseases: Rheumatoid arthritis, SLE, etc.
- Neoplasia

Causes of Increased ESR (Table 13.8)**Causes of Decreased ESR**

- Polycythemia vera.
- Sickle cell disease.
- Hypofibrinogenemia.

LE CELL TEST**Q. Write short note on LE cell.**

In autoimmune diseases (e.g. SLE, rheumatoid arthritis), variety of antibodies are found. One of them is an antinuclear antibody (ANA) which cannot penetrate intact cells. ANAs can react with nuclei of damaged cells and converts nuclear chromatin into homogeneous material. *In vivo*, ANAs act on the nuclei of damaged cells and denatures it into homogeneous material. This nuclear material is phagocytosed in the presence of complement by phagocytic leukocyte (neutrophil or macrophage). The LE cell (Refer

Figs 6.18A and B) is any phagocytic leukocyte (neutrophil or macrophage) that has engulfed the denatured nucleus of an injured cell. In the tissue they are known as LE bodies or hematoxylin bodies.

Tart cell: LE cell has to be differentiated from a tart cell (Refer Fig. 6.18C). It is a monocyte/neutrophil containing a phagocytosed nucleus of another cell. The nuclear material is not homogeneous and it retains its chromatin pattern. It may be found in healthy individuals.

Significance: LE cells may be demonstrated in SLE, rheumatoid arthritis, other autoimmune disorders, hepatitis and penicillin sensitivity.

BONE MARROW EXAMINATION

Bone marrow examination is essentially done to confirm or rule out a hematologic disorder. It also helps in evaluation of non-hematological disorders (e.g. metastasis). Bone marrow may be obtained by:

- **Aspiration:** Bone marrow aspiration is a simple, easy and safe procedure.
- **Trephine biopsy** is indicated in conditions where the aspiration either fails to yield marrow or to confirm some of the diseases (where biopsy findings are diagnostic).

Bone Marrow Aspiration

Bone marrow needles: Needles commonly employed for the aspirations of the marrow are Salah needle and Klima needle.

Sites for Bone Marrow Aspirate

Usual sites for bone marrow aspiration are as follows:

- Sternum.
- Posterior superior iliac spine.
- Iliac crest.
- Anterior superior iliac spine.
- Spinous process of lumbar vertebra.

In **infants**, upper end of the tibia is the ideal site for marrow aspirate.

Indications for bone marrow aspiration (Box 13.3).

Q. Write short note on indications/absolute for bone marrow aspiration.

Contraindications for bone marrow aspiration:

- Hemophilia.
- Congenital hemorrhagic disorders.

TABLE 13.3: Indications for bone marrow aspiration

Diagnostic
<ul style="list-style-type: none"> • Primary hematolymphoid disorders <ul style="list-style-type: none"> – Red cell disorders: Nutritional anemia (e.g. megaloblastic anemia), pure red cell aplasia – White cell disorders: Subleukemic/aleukemic leukemia, diagnosis and classification of acute leukemias – Megakaryocytic disorders: ITP and other thrombocytopenias – Myeloproliferative neoplasms: Polycythemia vera, chronic myeloid leukemia, idiopathic thrombocythemia – Myelodysplastic syndromes – Plasma cell neoplasms: Multiple myeloma, Waldenström macroglobulinemia • Systemic diseases: Storage disorders (e.g. Gaucher, Niemann–Pick disease) • Staging of lymphoid malignancies and solid tumors: Lymphoma, metastatic deposits (e.g. carcinoma prostate, breast, lung, kidney) • Detection of infection and/or source of PUO <ul style="list-style-type: none"> – Parasitic disorders: Kala-azar – Fungal disorders: Histoplasma – Mycobacterial infection • Iron store • Miscellaneous disorders: Pancytopenia or unexplained cytopenias, pyrexia of unknown origin
Post-treatment follow up: To know the response to therapy and follow up in cases of leukemia, aplastic anemia and agranulocytosis
Therapeutic: Bone marrow transplant

Dry Tap

Q. Write short answer on causes of dry tap in bone marrow aspiration.

During aspiration, if the marrow is not obtained it is called a dry tap. Dry tap is common in:

- Hairy cell leukemia.
- Myelofibrosis (marrow has been replaced by fibrous tissue).

Complications of bone marrow aspiration and/biopsy

- Local infection.
- Hemorrhage.
- Cardiac tamponade or mediastinitis.

Bone Marrow Trephine Biopsy

Marrow trephine biopsy is performed by one of the trephine biopsy needles like Jamshidi needle or Westerman–Jensen needle or Islam needle. The biopsy obtained consists of a core

of bone with marrow. These are excellent for morphological evaluation as well as for special stains. Trephine biopsy tissue is decalcified and processed like other histopathological tissue containing bone. Sections are stained with hematoxylin and eosin stain, reticulin stain, Masson's trichrome stain (for fibrous tissue). Immunocytochemical staining can be performed especially for acute leukemias.

Sites of Trephine Biopsy

- Posterior superior iliac spine (most commonly used site).
- Anterior superior iliac spine.
- Spinous process of vertebra.

Indications for Trephine Biopsy (Box 13.4)

Q. Write short note on indications for bone marrow trephine biopsy.

OSMOTIC FRAGILITY TEST

Q. Write short note on osmotic fragility test.

The normal red cell membrane is unstretchable and is freely permeable to water. RBCs are not lysed in normal buffered (pH 7) saline solution (9 g/L NaCl). The osmotic fragility test measures the ability of RBCs to withstand lysis when suspended in buffered solutions of various concentrations of sodium chloride (NaCl). The pattern of lysis helps to determine whether the shape of RBCs is normal or abnormal. Spherocytes and stomatocytes hemolyze with higher concentration of NaCl compared to normal red blood cells.

Principle

- In this test, small volumes of blood are mixed with large excess of buffered saline of varying concentration.

BOX 13.4: Indications for trephine biopsy

- Aplastic anemia
- Myeloproliferative neoplasms—to study reticulin fibrosis in myelofibrosis
- Myelodysplastic syndromes
- Pre- and post-bone marrow transplantation
- Pyrexia of unknown origin (granuloma of tuberculosis)
- Bone morphology in chronic renal failure, osteoporosis and osteomalacia
- Staging
 - Lymphoma
 - To detect metastasis in cancer patients
- AIDS

- When the red cells are suspended in buffered hypotonic solutions (i.e. the strength of the saline solution is decreased below normal saline), their volume progressively increases till a “critical hemolytic volume” is reached. At this critical point, the red cell membrane ruptures and liberates hemoglobin into the supernatant solution. The ability of normal RBCs to withstand hypotonic solution, lies in its biconcave shape which allows considerable water to enter the RBC before it stretches enough to break or lyse.
- If RBCs are spherocytic, there is loss of membrane which causes reduction in their surface area. The spherocytes cannot expand as much as normal biconcave RBCs and there is decrease in the critical hemolytic volume. When the strength of the saline solution is decreased (hypotonic) spherocytic red cells (which do not have normal biconcave shape) will begin to **hemolyze more than normal red cells**. The sooner hemolysis occurs, the greater the osmotic fragility of the cells. Incubation of blood overnight at 37°C makes the osmotic fragility test (OFT) more sensitive.

Interpretation

- **Normal:** Lysis of fresh RBCs begins at 0.5% of NaCl solution and is complete at 0.3%. It may be normal in patients with mild HS.
- **Increased osmotic fragility:** Spherocytes having decreased surface/volume ratio, have a limited capacity to expand in hypotonic solutions. Lysis of spherocytes begins early at a higher concentration of NaCl than do normal biconcave red cells. This is known as **increased osmotic fragility** and shows a **shift to right** of normal range in the graph (refer Fig. 10.15). Increased osmotic fragility indicates that the red cells are spherocytic. Increased osmotic fragility is observed in **hereditary spherocytosis, hereditary elliptocytosis** and other conditions associated with spherocytosis, like in **autoimmune hemolytic anemia and burns**.
- **Decreased osmotic fragility:** Conversely, hypochromic and flat red cells have an increased capacity to expand in hypotonic solutions. They lyse at a lower concentration than do normal cells, and thus have decreased **osmotic fragility**. The graph shows **shift to the left** of normal range and indicate that the red cells are flattened or hypochromic. This type of RBCs have increased surface/volume ratio and are found in **iron deficiency, thalassemia, sickle cell anemia, liver disease and reticulocytosis**.

Use

For the diagnosis of spherocytosis. However, it is time-consuming, tedious and cannot distinguish between the causes of spherocytosis.

LABORATORY EVALUATION OF HEMOSTATIC DISORDERS

Q. Discuss laboratory diagnosis in a case of bleeding disorder.

The clinical laboratory evaluation is an integral part of the diagnosis and management of patients with bleeding disorders. Laboratory tests for hemostatic disorders depend on the component of hemostasis that is involved (Table 13.9).

Most bleeding disorders are caused by one of three defects: (i) a defect in platelet number or function, (ii) a defect in platelet-vessel wall interactions (i.e. an abnormality in the adhesive interactions between platelets and the vessel wall) or (iii) a defect or deficiency in coagulation factor. The diagnosis of bleeding disorders requires a battery of tests (Table 13.10) which include screening tests done in all patients with history of bleeding, followed by specific tests to identify the exact nature of the disorder.

TABLE 13.9: Laboratory tests for hemostatic disorders depending on the component involved

Platelet component	Plate-vessel wall interaction	Coagulation component
<ul style="list-style-type: none"> • Platelet count • Platelet aggregation test • Clot retraction test 	<ul style="list-style-type: none"> • Capillary fragility test (Tourniquet test) • Bleeding time 	<ul style="list-style-type: none"> • Clotting time • Prothrombin time (PT) • Activated partial thromboplastin time (APTT) • Thrombin time (TT) • Prothrombin consumption time • Factor assay

TABLE 13.10: Laboratory tests in bleeding disorders

Screening tests	Accessory tests	Others
Platelet count	Capillary fragility test (Tourniquet test)	Prothrombin consumption time
Bleeding time	Clotting time	Factor assay
Prothrombin time (PT)	Clot retraction test	
Activated partial thromboplastin time (APTT)		
Thrombin time (TT)		

Tests for Platelet Component

Platelet Count

It is obtained on anticoagulated blood either manually or by electronic particle counters.

- **Normal range of platelet count:** 1, 50,000–4,50,000 platelets/cu mm.
- **Causes of thrombocytopenia** (refer Box 12.4).

Platelet Aggregation

These tests measure the ability of platelets to aggregate in response to agonists like thrombin and form the basis for qualitative tests of von Willebrand factor. They are used for classification of congenital qualitative disorders of platelets.

Clot Retraction Test

Q. Write short essay/note on clot retraction test and causes of impaired clot retraction.

After the coagulation of blood, the clot under the action of thrombasthenin (a substance released from platelets) undergoes contraction and starts retracting within one hour. The clot shows 50% retraction in 2–4 hours. This process is completed in 18–24 hours with separation of serum. Subsequently, the fibrin clot dissolves due to fibrinolysis and the RBCs sink to the bottom. Clot retraction is dependent on normal platelet number, platelet function, concentration of fibrinogen and the activity of the fibrinolytic pathway.

- **Normal value:** Normal clot retraction shows more than 50% of serum separated at the end of 24 hours. A normal clot is firm, rubbery, elastic and not easily broken.
- **Interpretation:** Absent or reduced clot retraction is seen in:
 - Fibrinogen deficiency (congenital or acquired).
 - Thrombocytopenia.
 - Thrombasthenia.

Tests for Platelet and Vascular Component

Capillary Fragility Test (Hess Test/Tourniquet Test)

Q. Write short essay/note on capillary fragility test (Hess test/Tourniquet test).

It measures the ability of capillaries to withstand the increased stress.

Procedure: Sphygmomanometer cuff is tied to the upper arm above the elbow and the cuff is inflated to 80 mm for 5 minutes. Release the pressure after 5 minutes. The number

of petechiae present in a circle of 5 cm diameter on the flexor aspect of forearm (below the bend of the elbow) is noted.

- **Normal:** 0–5 petechiae.
- **Interpretation:** Positive test is indicated by more than 10 petechiae and is found in:
 - Vessel wall abnormalities: Vascular purpura, scurvy.
 - Platelet disorders: Thrombocytopenia, defective platelet function.

Bleeding Time

Q. Write short essay/note on bleeding time, its definition and methods.

Bleeding time (BT) is used as screening test for disorders of platelet-vessel wall interactions. It measures the time required for bleeding to stop after a standardized superficial cut of the skin capillary bed.

Methods: Duke's method (obsolete), Ivy's method and template method (method of choice).

Template method for bleeding time

Template is a disposable blade fitted on to a holder made of plastic and is used for the test. The blade projects through the bottom so that the incision made through the slit is 9 mm long and 1 mm deep.

Principle: A small skin cut of a standard size and depth is made and the oozing blood is wiped with a filter paper. Bleeding stops when the capillaries contract and platelet plug seals the vessel.

Normal range: 2–9 minutes.

Uses: Tests for bleeding time is prone to problems of reproducibility, sensitivity and specificity. Though, it is one of the screening tests, it is not recommended as routine preoperative screening test. This test evaluates the defects of primary hemostasis. Thus, bleeding time measures the platelet-vessel wall interactions (integrity of capillary and platelet function).

Note: The bleeding time usually is not prolonged in patients with coagulation factor deficiencies.

Interpretation: Prolonged bleeding time is found in:

- **Platelet disorders:**
 - **Quantitative:** Thrombocytopenia. If platelet count is below 50,000/mm³, BT should not be performed as bleeding may be difficult to stop.
 - **Qualitative:** von Willebrand disease, Bernard-Soulier syndrome, Glanzmann's thrombasthenia.
- **Primary vascular disorders:** Ehlers-Danlos syndrome.
- **Platelet-vessel wall interactions:** von Willebrand disease.

- **Others:** Afibrinogenemia, severe hypofibrinogenemia, uremia, aspirin.

Tests for Coagulation Component

Coagulation or Clotting Time (Lee–White Method)

It measures the time taken for the fresh blood to clot.

Normal: 4–11 minutes.

Disadvantages

- **Not a sensitive test:** It is one of the oldest tests and is not sensitive as it fails to detect mild/ moderate coagulant defects. Hence, it is obsolete now and not recommended as a screening test. PTT is a more sensitive test for assessment of the coagulation cascade. Clotting time is prolonged only with severe deficiency of factor VIII, IX or fibrinogen (afibrinogenemia) and in heparin therapy.
- **Misleading:** Normal value may be obtained in mild-to-moderately severe hemophilia A and B and it does not exclude major factor deficiency.

Quick's One Stage Prothrombin Time

Q. Write short essay/note on prothrombin time.

Principle: Prothrombin time (PT) is the time taken by citrated plasma to clot after the addition of tissue thromboplastin and calcium. It tests *extrinsic and common pathway* of coagulation system.

Normal range: 11–16 seconds.

Reporting of prothrombin time: Prothrombin time may be reported in different ways:

- Patient PT and control PT in seconds.
- Ratio of patient PT to control PT.

Q. Write short note on INR.

International normalized ratio (INR): Due to the inherent variation in the sensitivity of thromboplastin reagents, it is advisable to report the PT in international normalized ratio (INR). It provides a uniform scale in spite of using different sources of thromboplastin. WHO recommended that each thromboplastin should have ISI (International sensitivity index) value. INR is calculated using the formula: $INR = (PT \text{ patient in seconds} / PT \text{ normal plasma in seconds})^{ISI}$.

Uses of Prothrombin Time

- **Screening test to evaluate coagulation disorders:** It measures coagulation factor I, II, V, VII and X. Deficiency of any one of these factors leads to prolongation of PT. It should be used along with PTT.

- **To monitor oral anticoagulant therapy.**

- **To evaluate liver function:** Liver disease can result in deficiency of the coagulation factors. Hence, PT should be performed before a *liver biopsy* and *prolonged* PT is a contraindication for liver biopsy.

Interpretation: Prolonged PT is seen in:

- Liver disease.
- Administration of oral anticoagulants like coumarin.
- Vitamin K deficiency: Obstructive jaundice, hemorrhagic disease of the newborn.
- Deficiency of factors I, II, V, VII and X.
- Disseminated intravascular coagulation (DIC).

Activated Partial Thromboplastin Time (Partial Thromboplastin Time)

Principle: Activated partial thromboplastin time (APTT) is the time taken for citrated plasma to clot in the presence of a surface activator (kaolin), phospholipid and calcium. Partial thromboplastin time (PTT) is a measure of the *intrinsic* and *common coagulation pathways*.

Normal range: 30–40 seconds.

Reporting: The patient's value is always to be reported with the control values in seconds. A prolongation of the patient value more than 8 seconds of the control value is considered as abnormal.

Uses

- **Best single screening test for coagulation disorders.** This test is abnormal with deficiencies of II, V, VIII, IX, X, XI and XII.
- For screening hemophilia A and B.
- For detecting coagulation inhibitors.
- For monitoring anticoagulant therapy like heparin.

Interpretation: Common causes of a prolonged APTT are as follows:

- Inherited coagulation disorders—deficiency of factor II, V, VIII, IX, X, XI, XII (e.g. hemophilia A, hemophilia B).
- von Willebrand disease.
- Disseminated intravascular coagulation.
- Heparin therapy.
- Vitamin K deficiency.
- Liver disease.
- Oral anticoagulant therapy.

Summary of screening tests for bleeding disorders are presented in Table 13.11.

Q. Write short essay/note on normal range for bleeding time, clotting time, prothrombin time (PT), activated plasma thromboplastin time (APTT).

TABLE 13.11: Summary of screening tests for bleeding disorders

Investigation	Normal range	Main causes of abnormal test
Platelet count	150–450 × 10 ⁹ /L	Thrombocytopenia
Bleeding time (template method)	2–9 minutes	Thrombocytopenia, abnormal platelet function, deficiency of von Willebrand factor, vascular abnormalities
Coagulation/clotting time (Lee–White method)—not a sensitive test	4–11 minutes	Severe deficiency of factor VIII, IX or fibrinogen (afibrinogenemia) and in heparin therapy
Prothrombin time (PT)	11–16 seconds	Deficiency of factor II, V, VII or X
Activated plasma thromboplastin time (APTT)	30–40 seconds	Deficiency of factor II, V, VIII, IX, X, XI, XII, heparin, antibodies against clotting factors, lupus anticoagulant

URINE ANALYSIS

Urine analysis (urinalysis) reflects the state of function of the kidneys and urinary tract. It provides information about metabolic or systemic (nonrenal) disorders. Urine examination should precede all other invasive/non-invasive diagnostic investigations for renal function.

Methods of Collection

Q. Write short essay/note on urine sample collection.

- **Midstream specimen:** This is used for all types of urine examination. After voiding first half of urine into the toilet, a part of the next voided urine is collected as midstream sample.
- **Clean-catch specimen:** This is the method of collection for culture and sensitivity of urine. The external genitalia are cleaned with soap and water and specimen is collected as mentioned in the midstream sample.
- **Catheter specimen:** It is used for culture and sensitivity in bed-ridden patients or patients with urinary tract obstruction.
- **Collection from infants:** It is usually collected by a clean plastic bag attached around the genitalia. For

bacteriological examination urine is aspirated from bladder by needle.

Preservation of Urine

Q. Write short essay/note on preservative for urine collection.

Urine sample should be **examined within 2 hours** of collection. If delay is likely to occur, it should be preserved by one of the following methods:

- Refrigeration without any preservative
- Use of preservatives like toluene (add few drops to form a thin layer on the urine surface), concentrated HCl (in the ratio of 1 mL for 125 mL of urine), thymol and formaldehyde (add 1 drop for 15 mL of urine- preserves cells and casts).

Examination of Urine

Urine examination consists of: (i) physical examination, (ii) chemical examination and (iii) microscopic examination.

Physical Examination

Q. Write short essay/note on physical examination of urine.

Volume

Q. Write short answer on volume of urine.

Q. Write short answer on oliguria.

A healthy adult excretes about 600–2000 mL of urine in 24 hours. In infants, the volume is 300–600 mL/day. Volume is measured by collecting 24-hour urine samples in a measuring cylinder.

- **Polyuria:** Increased urine output (more than 2 liters in 24 hours). Its causes include physiological (e.g. increased fluid intake) or pathological (e.g. diabetes mellitus, chronic renal diseases, diuretic therapy, diabetes insipidus, primary aldosteronism).
- **Oliguria:** Decreased urinary output (less than 500 mL in 24 hours). Its causes include restricted intake of fluid, excessive loss of fluid (e.g. in hemorrhage, burns, dehydration and shock) renal diseases (e.g. acute glomerulonephritis, nephrotic syndrome, acute tubular necrosis as in shock, burns, crush syndrome, incompatible blood transfusion and heavy metal poisoning) and Addison's disease.
- **Anuria:** Markedly diminished urine output, usually less than 125 mL in 24 hours. Causes include renal ischemia, tumors and renal stones.

Color (Table 13.12)

Normal urine is **straw to amber colored** due to the presence of urochrome pigment, excretion of which is generally proportional to the metabolic rate.

Q. Write short essay/note on chyluria.

Chyluria is a rare condition in which the **urine contains lymph**. **Obstruction to lymph flow** and rupture of lymphatic vessels into the renal pelvis, ureters, bladder or urethra may be associated with chyluria. The causes include **filariasis, abdominal lymphadenopathy and tumors**. The amount of lymph determines the color of urine which may range from clear to opaque or milky.

Lipiduria: In nephrotic syndrome, urine shows fat globules which are triglycerides (neutral fat) and cholesterol. It may also be observed in patients with bone fractures.

Specific Gravity**Q. Write short answer on specific gravity of urine including normal values and methods of estimation.**

Specific gravity depends on the number, density and weight of the solute particles in the urine. It is used as a measure of the concentrating power of the kidney. Normal specific gravity of a 24-hour urine sample is 1.003–1.035, average being 1.016. Specific gravity provides information about the renal status and hydration.

Methods: Specific gravity (SG) is measured by: (i) urinometer, (ii) refractometer and (iii) dipstick method. Urinometer and refractometer methods are more accurate as compared to the dipstick method.

TABLE 13.12: Conditions associated with color changes in urine

Color	Condition
Colorless	Dilute urine as in polyuria (e.g. diabetes mellitus, diabetes insipidus)
Dark brown	Oliguria
Smoky (red or red-brown)	Red blood cells (hematuria)
Cola colored	Intravascular hemolysis
Yellow-brown	Bilirubin
Orange- brown	Urobilin /urobilinogen
Dark colored on standing	Alkaptonuria (presence of homogentisic acid)
Milky	Pus or chyle (chyluria)

Q. Write short answer on causes of increased and decreased specific gravity of urine.

Causes of increased specific gravity: Glycosuria (e.g. diabetes mellitus), proteinuria (e.g. nephrotic syndrome), dehydration (e.g. restricted fluid intake, diarrhea, vomiting, fever and excessive sweating).

Causes of decreased specific gravity: Excessive fluid intake, diabetes insipidus and end-stage kidney (e.g. chronic glomerulonephritis, chronic pyelonephritis, bilateral polycystic kidneys and hypertension).

Q. Write short answer on low fixed specific gravity of urine and its importance.

Low and fixed specific gravity: When **specific gravity is fixed at 1.010**, this is known as **isosthenuria**. It is indicative of severe renal damage (chronic renal failure) with disturbance of both the concentrating and diluting abilities of the kidney.

Chemical Examination**Proteinuria****Q. What is proteinuria? Name the methods of its detection.**

The presence of detectable protein in the urine is known as **proteinuria**. It indicates glomerular injury. If turbid, filter or centrifuge the urine before testing.

Test for Protein in Urine (Box 13.3)**Q. Write short answer on tests for proteinuria.****BOX 13.3:** Tests for protein

1. Heat and acetic acid test
2. Sulfosalicylic acid test
3. Heller's test
4. Dipstick method

Heat and Acetic Acid Test

Principle: Heat-induced **coagulation of proteins** and precipitation. Coagulation can be further enhanced when drops of acetic acid are added.

Sulfosalicylic Acid Test**Q. Write short answer on sulfosalicylic acid test.**

- This test **detects all types of proteins** (albumin, globulin, glycoproteins and Bence Jones proteins).
- **Principle:** **Cold precipitation of proteins** by a strong acid. More sensitive and reliable than heat and acetic acid test.

- **Procedure:** Take 2.5 mL of urine in a small test tube. Slowly pour 2.5 mL of 3% sulfosalicylic acid. Wait for 10 minutes.
- **Interpretation:** Presence of a **cloudy precipitate** indicates the presence of proteins in urine. However, it also precipitates mucus and Bence Jones proteins.

Heller's Test

- **Principle:** Cold precipitation of proteins by a strong acid.
- **Procedure:** Take 5 mL of urine in a test tube and add a few drops of concentrated nitric acid.
- **Interpretation:** Presence of proteins is indicated by a **white ring** at the junction of urine and acid.
- **Precaution:** Filter urine if turbid as it interferes with the final reading.

Dipstick Method

- **Principle:** The test is based on the protein error of pH indicators. At a constant pH any color change in the indicator is due to protein. The reagent strip is impregnated with an indicator tetrabromophenol blue or tetrachlorophenol-tetrabromosulfophthalein buffered to pH 3.0. At this pH it is yellow in the absence of protein. If urine contains protein (which will elicit a pH change), it forms a complex with the indicator turning its color to green or bluish green.

Quantitative Estimation of Proteins in Urine

Q. Write short answer on Esbach's albuminometer.

Protein excretion in a 24-hour urine sample is required in suspected cases of nephrotic syndrome (>3.5 g/24 hours) and orthostatic/postural proteinuria. This may be carried out by Esbach's albuminometer method.

Principle: Cold precipitation of proteins by a strong acid.

Procedure: Fill the albuminometer with urine up to the mark U. Add Esbach's reagent up to the mark R. Stopper the Esbach's albuminometer, mix and allow it to stand for 24 hours. Take the reading from the level of precipitation in the albuminometer and divide the value by 10 to get the percentage of total proteins.

Categories of Proteinuria

- **Proteinuria quantification:** Classified depending on the amount of protein in the urine as **heavy** (>4 g/day), **moderate** (1–4 g/day) and **mild** (less than 1 g/day) proteinuria.
- **Qualitative categories of proteinuria:** Classified depending on the structure involved as (i) **renal** (glomerular causes/pattern, tubular cause/pattern), (ii) **prerenal** and (iii) **postrenal**.
- **Types of glomerular proteinuria.**

Q. Write short essay/note on selective proteinuria.

- **Selective proteinuria:** In this type, only intermediate-sized (<100 kDa) proteins (such as albumin, transferrin) leaks through the glomerulus.
- **Nonselective proteinuria:** It is characterized by leakage of range of different proteins including larger proteins (e.g. immunoglobulins) through the glomerulus.

Causes of Proteinuria (Box 13.5)

Q. Write short note/answer on causes of proteinuria.

Bence Jones Proteins

Bence Jones proteins are light chains of immunoglobulins, secreted in **multiple myeloma**. It may also be found in macroglobulinemia and lymphoma. Bence Jones proteins precipitate at temperature between 40°C and 60°C , and redissolve near 100°C . It reappears on cooling to 40°C – 60°C .

Microalbuminuria

Screening tests for urine protein are not sensitive enough to detect very small quantities of protein. Microalbuminuria is the presence of albumin in urine above the normal level but below the detectable range of conventional methods. It is defined as the **persistent elevation of the urinary albumin excretion of 20–200 mg/L** (or 20–200 micrograms/min) in an early morning urine sample. It indicates early and possibly reversible glomerular damage.

Causes of microalbuminuria

- **Diabetes mellitus:** In diabetic patients, presence of microalbuminuria is associated with **increased cardiovascular mortality** and is a risk factor for **renal mortality**. Early detection can predict the development of renal complications in diabetics.
- **Essential hypertension:** In hypertensive patients, microalbuminuria **predicts cardiovascular morbidity and mortality**.

Reducing Substances in Urine

Reducing substances are those compounds which reduce cupric ions (from copper sulfate in Benedict's reagent) in an alkaline solution to cuprous ions (cuprous oxide). Such substances may be sugar or nonsugar.

- **Sugar:** These include: Glucose, fructose, pentose, galactose, lactose, pentose and maltose. Sucrose gives negative result with Benedict's test and glucose oxidase. Special tests are required to differentiate the sugar occurring in urine.
- **Nonsugar:** Ascorbic acid, uric acid, urates, glucuronides, chloroform, formaldehyde, salicylates, streptomycin,

BOX 13.5: Causes and amount of proteinuria**1. Renal**

- A. **Glomerular damage:** Due to increased permeability of glomerular capillary wall
- **Heavy proteinuria:** Nephrotic syndrome
 - **Moderate to heavy:** Acute rapidly progressive glomerulonephritis, chronic glomerulonephritis, malignant hypertension, SLE
 - **Mild to moderate:** Acute glomerulonephritis, nephrosclerosis
- B. **Tubular damage:** Inability to absorb low molecular weight proteins by tubules
- **Moderate:** Heavy metal and vitamin D intoxication
 - **Mild:** Acute and chronic pyelonephritis, acute tubular necrosis, polycystic kidney disease, hypokalemia

2. Prerenal

- A. **Overflow proteinuria:** Develops due to the overflow of excess levels of a protein in the circulation. In this protein in the urine may be hemoglobin, myoglobin, or immunoglobulin
- **Immunoglobulin: Moderate proteinuria** in plasma cell dyscrasias (Bence Jones protein-light chain of immunoglobulin)
 - **Hemoglobin:** in intravascular hemolysis
 - **Myoglobin:** in skeletal muscle trauma
 - **Lysozyme:** Acute myeloid leukemia M4 or M5
- B. **Hemodynamic proteinuria:** Due to alteration of blood flow through glomeruli
- **Mild asymptomatic proteinuria:** After excessive exercise, postural (orthostatic) albuminuria, congestive cardiac failure, high fever, exposure to cold, dehydration

3. Postrenal

- Inflammatory or neoplastic lesions of renal pelvis, ureter, bladder, prostate or urethra

phenol, PAS, homogentisic acid (alkaptonuria) and creatinine.

Blood glucose level varies between 70 and 120 mg/dL. This may increase to 120–160 mg/dL after a meal. Normally, all the glucose in the blood is filtered through glomerulus and reabsorbed at proximal tubules. If the renal threshold (the lowest blood glucose level that will result in glycosuria) is exceeded (usually greater than 180–200 mg/dL), the excess glucose will not be reabsorbed into the blood and will be eliminated in the urine as in cases of diabetes mellitus. The presence of detectable amounts of **glucose in urine** is termed **glycosuria**.

Tests for Reducing Substance (Box 13.4)

Q. Write short note on test for detection of glycosuria.

BOX 13.4: Tests for reducing substances in urine

1. Benedict qualitative test
2. Dipstick method

Benedict Qualitative Test (Semiquantitative)

Q. Write short note on principle of Benedict test.

This test detects the presence of reducing substances in urine and is not specific for glucose.

Principle: The copper sulphate present in the Benedict's reagent reacts with the **reducing substances** in the urine which **convert cupric sulphate to cuprous oxide** in hot alkaline media. Thus, this test is based on the reduction of cupric ions in Benedict's solution to cuprous ions. In the absence of reducing substances in urine, the color of the reagent remains blue.

Procedure: Take **5 mL of Benedict's** (qualitative) reagent in a test tube. Boil to exclude presence of reducing substance in reagent. Add **8 drops (0.5 mL) of protein-free urine**. Boil the mixture for 5 minutes and allow to cool. The **ratio of 5 mL Benedict's reagent and 8 drops (0.5 mL) of urine is important because it is a semiquantitative test**. Note the color of the precipitate which is cuprous oxide formed due to reduction of cupric sulphate of Benedict's reagent.

Interpretation: The change of color from blue to green, yellow and orange/red depends on the amount of sugar present.

Dipstick Method

Principle: Diastix/multistix/dipstix contains: (i) Glucose oxidase (ii) peroxidase (iii) chromogen: O-toluidine (clintix) or potassium iodide (multistix/diastix). Glucose present in the urine is oxidized by atmospheric oxygen in the presence of glucose oxidase to gluconic acid and hydrogen peroxide. The hydrogen peroxide, in the presence of peroxidase, oxidizes the reduced chromogen present in the dipstick to various shades of purple (oxidized chromogen). The color change depend on the amount of glucose (semiquantitative) present in the urine.

Cause of Glycosuria (Box 13.6)

Q. Write short note on causes of glycosuria.

The substances which may give positive reaction with Benedict's test but negative with glucose oxidase strips are

BOX 13.6: Causes of glycosuria**Glycosuria with hyperglycemia**

- Endocrine disorders
 - **Diabetes mellitus**
 - Acromegaly
 - Cushing's syndrome
 - Hyperthyroidism
 - Hyperadrenocorticism
 - Functioning α - or β -cell pancreatic tumors
 - Pheochromocytoma
- Non-endocrine diseases
 - Increased intracranial tension (brain tumor or hemorrhage)
 - Liver disorders
 - Drugs: Corticosteroids, adrenocorticotropic hormones, thiazides
 - Alimentary glycosuria

Glycosuria without hyperglycemia

- Renal glycosuria
- Pregnancy

nonsugar substances, such as ascorbic acid, glucuronic acid, homogentisic acid, salicylates, phenylketonuria and tyrosinemia.

Renal glycosuria: Renal glycosuria is a benign condition due to a reduced renal threshold for glucose. It is unrelated to diabetes and not accompanied by the classical symptoms of diabetes. Therefore, it should not be mistaken as diabetes mellitus.

Alimentary glycosuria: In certain individuals, blood glucose level may rapidly increase after meals resulting in its spill over into urine. This condition is known as alimentary (lag storage) glycosuria. It may be seen in some normal individuals and some patients with hepatic diseases, hyperthyroidism and peptic ulcer.

Fructosuria: Presence of fructose in urine. It is detected by Seliwanoff's test. *Causes* include high intake of fruits like grapes and oranges.

Lactosuria: Presence of lactose in urine. It is detected by Rubner's test. *Causes*—suckling infants and nursing mothers.

Pentosuria: Presence of pentose in urine. It is detected by Bial's test. *Cause*—ingestion of fruits.

Ketone Bodies

Q. Write short note on ketone bodies found in urine and tests for its detection.

The presence of ketone bodies in the urine is a measure of the metabolic rather than renal function. Whenever there is a

defect in carbohydrate metabolism, the fat is used as a source of energy. When increased quantities of fat are metabolized, there is increased production of ketone bodies which begin to accumulate in the blood and are subsequently excreted in the urine.

Ketone bodies are three metabolically related compounds:

- **Acetoacetic (diacetic) acid.**
- **β -hydroxybutyric acid.**
- **Acetone.**

If urine is left at room temperature, acetoacetic acid slowly converts into acetone.

Tests for Ketone Bodies (Box 13.7)**BOX 13.7:** Tests for ketone bodies

1. Rothera's test
2. Gerhardt's test (ferric chloride test)
3. Hart's test
4. Dipstick method

Rothera's Test

Q. Write short note on Rothera's test.

Principle: Acetoacetic acid (diacetic acid) and acetone react with sodium nitroprusside in presence of an alkali to form a purple color compound.

Procedure: Take 4 mL of urine in a test tube. Add a few crystals of sodium nitroprusside and saturate the urine with ammonium sulfate by mixing vigorously. Overlay with few drops of liquor ammonia along the wall of the tube.

Interpretation: Development of a **purple ring** indicates the presence of **acetoacetic acid/acetone** or both. A brown or red color is of no significance.

Gerhardt's Test (Ferric Chloride Test)

This is neither a very specific nor a sensitive test. The test **detects acetoacetic acid.**

Principle: Acetoacetic acid reacts with ferric chloride to give a deep red color.

Hart's Test

This test detects **β -hydroxybutyric acid**, which the above two tests fail to detect. **β -hydroxybutyric acid** is usually accompanied by acetoacetic acid.

Principle: **β -hydroxybutyric acid** is converted into acetone which reacts with sodium nitroprusside and liquor ammonia to give purple red color.

Dipstick Method

Principle: Dipstick contains buffers and sodium nitroferri-cyanide, which react with acetoacetic acid in the urine to form a pink-maroon color in 15 seconds. The strips detect acetoacetic acid and not acetone. However, acetone can be detected if glycine is incorporated into it.

Causes of Ketonuria (Box 13.8)

Q. Write short note on causes of ketonuria. List the non-diabetic causes.

BOX 13.8: Causes of ketonuria

Diabetic ketonuria

- Diabetic ketoacidosis

Nondiabetic ketonuria

- Starvation
- Prolonged vomiting or diarrhea
- Infant and children
 - Prolonged febrile illness
 - Toxic states accompanied by vomiting or diarrhea
 - Glycogen storage disorders (von Gierke's disease)
- Hyperemesis of pregnancy

Lactic acidosis

- Shock, diabetes mellitus, renal failure, liver disease, infection, and drugs (e.g. phenformin and salicylate poisoning)

Bilirubin (Bile Pigment)

Q. Write short note on bile pigments in urine.

Tests for bilirubin in urine provide information concerning metabolic or systemic disorders, especially liver function. Bilirubin is a breakdown product of hemoglobin and is normally not present in urine. Even trace amounts are clinically significant and only conjugated bilirubin is found in urine. Bilirubinuria causes yellow-brown to greenish-brown urine and forms yellow foam on shaking. Bilirubin is found only in freshly voided urine which upon standing is oxidized to biliverdin.

Tests for Bilirubin (Box 13.9)

BOX 13.9: Tests for bilirubin

1. Fouchet's test
2. Dipstick method

Fouchet's Test

- **Principle:** Fouchet's reagent contains **trichloroacetic acid and ferric chloride**. In an acidic medium ferric

chloride oxidizes **bilirubin** to produce a dark green colored **biliverdin**.

Dipstick Method

- **Principle:** The test is **based on a diazo reaction**. Bilirubin in the urine couples with a diazotized dichloroniline (content of strip) in a strongly acidic medium to form colored compound namely azobilirubin.

Causes of Bilirubinuria

- **Obstructive jaundice:** Urine shows bilirubin without urobilinogen. Bilirubin is of conjugated type.
- **Hepatocellular jaundice:** In acute viral hepatitis, bilirubin appears in the urine before the jaundice is clinically apparent. In a patient with pyrexia of unknown origin bilirubinuria indicates hepatitis.
- **Hemolytic jaundice: Bilirubin is absent in urine.** This is because in hemolytic anemia the hyperbilirubinemia is due to unconjugated bilirubin which is not water insoluble. Hence, not excreted in the urine.

Urobilinogen

Q. Write short note on urobilinogen in urine.

Urobilinogen is normally present in urine in trace amount (1–2 mg/dL) and is insufficient to cause a significant positive reaction. Whenever the liver is unable to efficiently remove the reabsorbed urobilinogen from the portal circulation (e.g. liver diseases, hemolytic anemia) more urobilinogen than normal is routed through the kidney and hence, excreted in the urine.

Tests for Urobilinogen (Box 13.10)

BOX 13.10: Tests for urobilinogen

1. Ehrlich's test
2. Dipstick method

Ehrlich's Test

- **Principle:** Ehrlich's reagent reacts with urobilinogen and forms a pink-colored aldehyde complex.
- **Interpretation:** Pink to cherry-red color indicates presence of urobilinogen.

Dipstick Method

- **Principle:** This method is based on the Ehrlich's test. The strip is coated with p-amino benzaldehyde with a color enhancer. It reacts with urobilinogen in a strongly acidic medium to produce a pink-red colored compound. The

color is matched with the color chart on the container of the dipsticks.

Causes of Increased Urobilinogen in Urine

Q. Write short note on urobilinogen in urine or in jaundice.

Urobilinogen is normally excreted in the urine in traces.

- **Hemolytic anemias** (without bilirubin in urine):
Thalassemia, sickle cell anemia, hereditary spherocytosis.
- **Liver diseases:** Preicteric phase of infective hepatitis, drugs or toxic hepatitis, cirrhosis.

Causes of Decreased/Absent Urobilinogen in Urine

In **obstructive jaundice**, bilirubin does not reach the intestine and hence, is not converted into urobilinogen.

Bile Salts

Q. Write short note on bile salts in urine and test for its detection.

Bile salts are composed of mixture of bile acids and glycine or taurine. Two important bile salts are sodium and potassium salts of glycocholate and taurocholate. Normally, bile salts are not present in urine.

Hay's Sulfur Test

Principle: Bile salts have unusual property of **lowering the surface tension of urine** markedly even when present in small concentrations. This property is made use of in the Hay's test.

Interpretation: Sulfur powder sinks to the bottom of test tube in the presence of bile salts in urine.

Causes: Hepatocellular and obstructive jaundice.

Tests for Blood in Urine

Q. Write short note on tests for blood in urine.

These tests detect hematuria, hemoglobinuria or myoglobinuria.

Hematuria: Presence of abnormal number of red cells in urine, e.g. renal stones, renal cell carcinoma.

Hemoglobinuria: Presence of free hemoglobin in urine, e.g. intravascular hemolysis.

Myoglobinuria: Presence of myoglobin in urine, e.g. crush injury to muscle, strenuous exercise.

Tests for Blood (Box 13.11)

BOX 13.11: Tests for blood

1. Benzidine test
2. Orthotoluidine test
3. Dipstick method

Benzidine Test

Q. Write short answer on principle of benzidine test.

- **Principle:** The test depends upon the ability of **heme compounds** derived from hemoglobin to **catalyze the oxidation of benzidine by hydrogen peroxide**.
- **Procedure:** Dissolve a small amount (knife-point full) of benzidine in 2 mL of glacial acetic acid and add an equal volume of 3% hydrogen peroxide. From this, take 2 mL in another test tube and add 2 mL of previously boiled and cooled urine and mix.
- **Control for the test:** If the test is negative, add a drop of blood to the above test tube. It should give blue color. This is to confirm the potency of reagents especially hydrogen peroxide and excludes a false negative reaction.
- **Interpretation:** The appearance of **blue color** indicates the presence of blood.
- **Precaution:** Presence of hypochlorite (bleach) and microbial peroxidase can cause false positive results. **Benzidine is carcinogenic.**

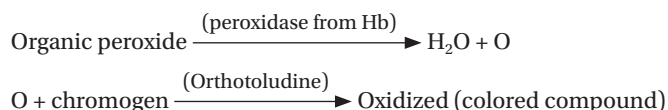
Orthotoluidine Test

- **Principle:** Peroxidase-like activity of heme present in hemoglobin **liberates oxygen from hydrogen peroxide** in the reagent. The liberated **oxygen changes the color of orthotoluidine**.
- **Interpretation:** The appearance of blue or green color indicates the presence of blood.

Dipstick Method

Very small amount of hemoglobin/RBCs are detected by dipstick method.

Principle: The test is based on the **liberation of oxygen from peroxide in the reagent strip by peroxidase-like activity of heme** present in hemoglobin. The liberated oxygen changes the color of the chromogen. The reagent area is impregnated with organic peroxide and the chromogen is orthotoluidine/tetramethylbenzidine.



Causes of Hematuria

Q. Write short note on causes of hematuria.

Urine is red colored in severe hematuria and smoky in mild hematuria. RBCs are demonstrable in urinary sediment. Causes of hematuria are shown in Box 13.12.

Hemoglobinuria

Hemoglobin is too large to pass through the glomerular filter. If the renal threshold is exceeded the hemoglobin can pass into the urine which becomes cola colored.

Interpretation: If the supernatant is colored pink, it indicates hemoglobinuria.

Note: Hemoglobin is precipitated when the urine is 80% saturated with ammonium sulfate but myoglobin is not.

Causes of hemoglobinuria: Any cause of hemolysis may cause hemoglobinuria and the presence of hemoglobinuria indicates significant intravascular hemolysis. These include paroxysmal nocturnal hemoglobinuria (PNH), paroxysmal cold hemoglobinuria (PCH), incompatible blood transfusion,

BOX 13.12: Causes of hematuria

Renal diseases

• Glomerular causes

- Acute glomerulonephritis
- Renal infarct
- Bacterial endocarditis with kidney involvement
- Malignant hypertension

• Nonglomerular causes

- Polycystic kidneys
- Renal cell carcinoma
- Renal stones (nephrolithiasis)
- Trauma (including renal biopsy)
- Renal tuberculosis
- Hydronephrosis

Lower urinary tract/prostatic diseases

- Bladder stones (lithiasis)
- Cystitis
- Urethritis
- Carcinoma bladder
- Carcinoma prostate

Blood disorders

- Bleeding disorders—coagulation disorders, severe thrombocytopenia
- Acute leukemia
- Sickle cell disease

Miscellaneous

- Instrumentation of urinary tract

severe burns, autoimmune hemolytic anemia (AIHA), march hemoglobinuria, snake bite, blackwater fever (falciparum malaria).

Multistix reagent strips for urine testing: These have single/multiple discrete cellulose squares which are impregnated with reagents for testing glucose, protein, pH, specific gravity, hemoglobin, ketone bodies, bilirubin and urobilinogen. There are single test strips, e.g. diastix for glucose and multistix for multiple tests.

Automated urinalysis: Fully automated urine analyser provided with automatic functions are presently available, e.g. Uriplus.

Microscopic Examination

Q. Write short note on microscopic examination of urine.

Microscopic examination of urinary deposit or sediment is an essential part of urine examination. The deposits are divided into two main groups; (i) organized deposits and (ii) unorganized deposits.

Organized Deposit

Organized deposit consists of: (i) **cells** [blood cells (red cells and white cells) epithelial cells (renal, transitional, and squamous)], (ii) **casts** (with or without inclusions), (iii) **crystals** and (iv) **other** abnormal cells or formed elements.

Cells

They are expressed as number of cells per low-power or high-power field.

1. **Red blood cells (RBCs):** Presence of RBCs (more than 2/hpf) in the urine indicates bleeding at any point in the urinary system from the glomerulus to the urethra. In **glomerular diseases**, the urine show **red cells with cellular protrusions or fragmentation** and are named as **dysmorphic** (distorted morphology) red blood cells.
2. **White blood cells (WBCs):** Increased number of WBCs (mainly neutrophils more than 5/hpf) in urine is known as **pyuria**. It is indicative of **urinary tract infection**. The causative organism of infection may be identified by bacteriological examination. When accompanied by **leukocyte casts** or mixed leukocyte-epithelial cell casts, increased urinary leukocytes are considered to be of **renal origin**.

Causes of pus cells in urine (pyuria): Pyelonephritis, urethritis, cystitis, urinary tract infection (UTI).

Glitter cell is a swollen neutrophil with its cytoplasmic granules showing constant Brownian motion. This

results in a shining or glittering appearance of the cell. Causes of glitter cell include dilute or hypotonic urine, chronic pyelonephritis and lower urinary tract infections.

3. **Epithelial cells:** These are derived from the urinary tract (transitional and renal) or genital tract (squamous). A few (0–2/hpf) transitional cells from the bladder may be present in the normal urine and squamous cells from the vulva and vagina usually contaminate a routine specimen from women.

Malignant cells may be seen in tumors of urinary bladder.

Casts (Box 13.13)

Q. Write short note on urinary casts and crystals.

They are one of the organized elements which are **formed only in the kidney** and are **indicative of a renal disease**. They are formed by solidification of **Tamm Horsfall protein**, a glycoprotein secreted in the distal convoluted tubules and collecting tubules. These proteins form a fibrillar meshwork (basic matrix) and can trap any elements including cells, cell fragments or granular material. **Casts** are cylindrical structures with **parallel sides** and rounded ends. They vary in size, shape and appearance. **Cylindroids** probably represent abortive casts. They have one tapering end while the other end is round and the sides are not parallel. The casts may have only proteins (hyaline and waxy casts) or have trapped granular debris (granular casts), epithelial cells (epithelial casts), leukocytes (leukocyte casts), red blood cells (RBC casts) or fat droplets (fatty casts).

BOX 13.13: Classification of casts

- **Matrix:** Hyaline, waxy
- **Cells:** RBCs and its remnants, leukocytes (neutrophils, lymphocytes), renal tubular epithelial cells, mixed cells (RBCs, neutrophils, and renal tubular cells)
- **Inclusions:** Granules (proteins, cell debris), fat globules (triglycerides, cholesterol esters), hemosiderin granules
- **Pigments:** Hemoglobin, myoglobin, bilirubin

Crystals

These are not usually present in urine. Crystals of oxalates, urates and cystine are present in patients with history of renal stone, while urates alone are present in *gout*. Oxalates, urates and cystine are present in acidic urine while phosphates, calcium carbonates and ammonium urates are present in alkaline urine.

Unorganized Sediments

Unorganized sediments consist of crystalline or amorphous material, the nature of which varies according to pH of the

urine (acid or alkaline). Majority of these have little diagnostic or prognostic significance.

BODY FLUIDS

Body fluids are lubricating fluids present within the body cavities. Normally, a small amount of fluid is present within the body cavities which keep the surfaces moist and lubricated so that the movement of the adjacent or the opposing membrane surfaces occur with minimal friction. Increase in the volume of the fluid in these cavities is known as **effusion**. The commonly examined body fluids in the laboratory include **pleural, pericardial, peritoneal and synovial fluid**.

The effusion may be broadly divided into **transudate** and **exudate**; the differences between them are listed in Table 2.3.

Specimen Collection

The body fluid is collected in a clean, dry container under aseptic precautions and atraumatically to avoid mixing with fresh blood. The fluid is collected in the following three sterile test tubes:

- Chemical examination: Fluoride tube.
- Microscopic examination: EDTA tube.
- Bacteriological examination: Plain tube (without anticoagulant).

They should be examined as early as possible to prevent chemical changes, growth of bacteria and disintegration of cells.

Examination of Body Fluids

Physical Examination

Note: volume, color and appearance.

Color: Pleural, pericardial and ascitic (peritoneal) fluids are usually clear and straw colored.

- Uniform **blood** stained fluid suggests malignancy involving the organs/tissues surrounding the respective body cavity.
- **Turbid** fluid may be due to high cell count or high protein content.
- **Chylous** with milky appearance usually indicates high lipid content due to lymphatic obstruction.

Transudate VS. exudate: It is important to differentiate whether the fluid is a transudate or exudate (refer Table 2.3).

- **Transudate** is usually seen in all body cavities with diseases like heart failure and hypoalbuminemic conditions (e.g. nephrotic syndrome). Cirrhosis results in prominent ascites, but may also cause pleural effusion.
- Exudate usually suggests infection or malignancy.

Chemical Examination

- **Protein estimation:** This helps to differentiate transudate from exudate.
- **Glucose estimation:** Low glucose in the body fluids usually suggests bacterial infection (including tuberculosis), malignancy or nonspecific inflammation.
- **Measurement of amylase in ascitic fluid** in patients with pancreatic lesions.

Microscopic Examination

- **Cell count** is done similar to total WBC count using improved Neubauer chamber. Normally, few mesothelial cells (lining cells of body cavities) and lymphocytes are seen.
- **Differential WBC count:** Centrifuge the body fluid and from the sediment prepare the smears (at least 2). **Stains** employed includes: 1) **Leishman's stain:** Stain one smear with Leishman's stain and count 100 cells and express the differential count and 2) **Gram's stain/acid-fast stain:** These stains are useful in suspected cases of infective/tubercular infections.
- **Cytological examination for malignant cells:** The smears are stained by Papanicolaou stain. Hematoxylin and eosin stain or Giemsa may also be used. Cytospin is a better alternative for making smears.

Microbiological Examination

Culture is done to identify the organism in cases of effusion due to infections.

CEREBROSPINAL FLUID EXAMINATION

Cerebrospinal fluid (CSF) is formed within the ventricles and circulates in the subarachnoid space (between arachnoid and pia matter) and in the ventricles. The total volume of CSF in adults ranges from 90–150 mL. It is a medium for transfer of substances from brain and spinal cord into the blood.

Importance of CSF examination: Analysis of the CSF is of diagnostic importance in conditions like meningitis or primary/metastatic tumor of CNS with CSF involvement.

Collection of CSF: CSF is usually obtained by lumbar puncture (LP) using an LP needle under strict aseptic conditions.

Lumbar puncture needle: The needle measures 10–12 cm in length. In children, a shorter needle is used. It has a needle and a stylette. The stylette has a pin which fits into the slot of the head of the needle and helps to keep the needle patent.

Sites

- **Lumbar puncture:** In adults, CSF is normally collected in the midline of the lower back in the **3rd lumbar space** and in children in the 4th lumbar space.
- **Cisternal puncture:** It is done in spinal cord block, vertebral deformity or infections in the tissues where lumbar puncture is usually done.
- **Ventricular puncture:** It is performed in infants who have open fontanella.

Method of Collection

Normally CSF is collected in three/four sterile test tubes. The amount of CSF collected should not exceed 6–8 mL. Tube 1 for estimation of protein and glucose and serology, tube 2 is used for preparation of smears to stain with the Gram stain or other stains and for culture and sensitivity, tube 3 for cell counts and differential counts and tube 4 (if indicated) for special tests, such as the cryptococcal antigen, serologic test for syphilis, molecular tests or other serologic studies and cytology.

Indications for Lumbar Puncture (Box 13.14)

Q. Write short note on indications for lumbar puncture.

BOX 13.14: Indications for lumbar puncture

Diagnostic indications

- Infection
 - Meningeal infection:
 - ◆ Bacterial
 - ◇ Pyogenic
 - ◇ Tuberculosis
 - ◇ Syphilitic: To differentiate general paresis of insane, tabes dorsalis and meningeal syphilis
 - ◆ Viral
 - ◆ Fungal
 - Encephalitis
- Subarachnoid hemorrhage
- Primary or metastatic malignancy (e.g. acute leukemia, lymphoma)
- Demyelinating diseases: Multiple sclerosis and subacute sclerosing panencephalitis+ (SSPE), Guillain Barre syndrome
- Spinal canal blockage leading to elevated intracranial tension (spinal cord tumors)
- For injecting the radio-opaque dye for myelography

Therapeutic indications

- Spinal anesthesia
- Intra-theal injection of chemotherapeutic drugs for CNS prophylaxis/relapse of ALL, lymphomas

Contraindications for Lumbar Puncture (Box 13.15)

BOX 13.15: Contraindications for lumbar puncture

- Raised intracranial pressure
- Local infective lesion
- Disseminated sclerosis
- Brain tumor
- Cardiorespiratory compromise
- Bleeding diathesis which is not been corrected

Complications of Lumbar Puncture (Box 13.16)

BOX 13.16: Complications of lumbar puncture

- Herniation of cerebellum through the foramen magnum due to raised intracranial pressure
- Hematoma, either extradural or subdural
- Introduction of infection by the LP needle through the infected skin or subcutaneous tissue
- Post-puncture headache
- Failure to obtain CSF (dry tap).

Examination of CSF

Physical Examination

CSF Pressure

The normal CSF pressure in adults is 90–180 mm of water in the lateral position, while in infants and children it ranges from 10 to 100 mm of water reaching the adult level by 6–8 years.

- **Causes of raised CSF pressure:** Meningitis, cerebral edema, mass lesions in brain, etc.
- **Causes of decreased CSF pressure:** Dehydration, circulatory collapse, etc.

Color and Appearance

- Normal CSF is clear and watery.
- Turbidity of CSF is due to pus or RBCs.
- **Xanthochromia** (yellow CSF) results from: (i) Old hemorrhage, (ii) obstructive jaundice, (iii) excess of protein, (iv) Froin syndrome.

Differentiation of subarachnoid hemorrhage from a traumatic puncture: The presence of blood in CSF may be due to trauma during lumbar puncture or subarachnoid hemorrhage.

- In traumatic tap, first few drops are hemorrhagic and subsequent ones are clear, while in subarachnoid hemorrhage blood is uniformly mixed with CSF.
- After centrifugation, the supernatant fluid is clear with a traumatic tap, whereas the supernatant appears xanthochromic (a faint pink, orange or yellow color caused by release of hemoglobin from hemolyzed red blood cells) in subarachnoid hemorrhage.

Clot Formation

Normal CSF does not clot. When blood-brain barrier is disturbed, fibrinogen appears in CSF. Fibrinogen gets converted to fibrin and forms clot.

Causes of Fibrin Clot

- Meningitis: In tuberculous meningitis, the clot is fine, delicate and typically described as cob-web appearance. In purulent meningitis, large clot is formed.
- Tumors of CNS.
- Polyneuritis.

Microscopic Examination

Total cell count: Normal CSF usually contains no cells, although cell count of 0–5 lymphocytes/L is considered as normal. An **increased cell count** is known as **pleocytosis**. Total cell count should be done immediately on undiluted CSF, since after a few hours, pus cells stick to each other and to the sides of the tube or degenerate. Cell counts are performed in a manual counting chamber, either in a Fuchs-Rosenthal or improved Neubauer chamber.

Significance

Neutrophils are increased in acute pyogenic meningitis. Lymphocytes are increased in viral, syphilitic, tuberculous and fungal meningitis.

Increased cell count should always be confirmed by bacterial or serological tests. India-ink preparation is used for diagnosis of cryptococcal meningitis. In tuberculous meningitis, acid-fast stain can detect tuberculous bacilli.

Biochemical Evaluation

Proteins are elevated in meningitis and glucose level is reduced due to utilization by the microbes. These changes are more marked in pyogenic meningitis.

Chloride reduction in tuberculous meningitis is due to general chloride deficiency because of dehydration rather than any specific effect of mycobacteria.

Refer chapter 28 for CSF changes in various meningitis.

SEMEN ANALYSIS

Q. Write short note on semen analysis.

Semen (seminal fluid) consists of spermatozoa (sperms) and the fluid part. About 40% cases of infertility are due to abnormalities in semen and therefore, semen analysis is the first test to be performed while investigating for infertility. Defect of sperms may be **quantitative** (absence of sperms, lack of enough sperms) or **qualitative**.

Indications for semen analysis

- Assessment of fertility/infertility.
- Determine the **effectiveness of vasectomy**.
- Determine the **suitability of semen for artificial insemination**.
- **Medicolegal purpose**: In alleged rape cases, vaginal pool smears are examined to detect sperms.
- **For selection of assisted reproductive technology** (e.g. in vitro fertilization, gamete intrafallopian transfer technique).

Collection of the Sample

Q. Write short note on collection of semen for analysis.

Patient is asked to collect the semen by masturbation after a minimum of 2 days and a maximum of 7 days of sexual abstinence. Specimen should be collected in a clean, dry, wide-mouthed plastic/glass container. Collection of condom sample is not advisable because they often contain spermicidal agents which impair the sperm motility.

Examination of Semen

Q. Write short note on various test/parameters of the semen with normal range.

Physical Examination

- **Liquefaction**: Immediately after ejaculation, the semen is normally a semisolid coagulated mass. At room temperature, the semen usually begins to liquefy (become thinner) within a few minutes and completely liquefies within 15 minutes. Within 30 minutes it becomes more homogeneous and watery.
- **Semen viscosity**: Fresh semen is fairly viscid and the viscosity can be estimated by gently aspirating semen into a wide-bore (approximately 1.5 mm diameter) plastic disposable pipette, allowing the semen to drop by gravity. Normal semen falls drop by drop and if viscosity is abnormal, the drop will form a thread more than 2 cm long. Normal viscosity is important, since increase in viscosity affects sperm motility.

- **Appearance**: Freshly ejaculated semen is an opaque, white gray and viscid fluid. After liquefaction it has homogeneous grey-opalescent appearance. Semen may have red-brown color when red blood cells are present (hemospermia) or yellow in patients with jaundice or ingestion of certain vitamins or drugs.
- **Semen volume**: Normal volume ranges from 1.4–1.7 mL per ejaculate. Low semen volume may be due to obstruction of the ejaculatory duct, congenital bilateral absence of the vas deferens or can also be due to difficulty in collection. High semen volume may be due to active exudation in cases of inflammatory lesions of the accessory organs.
- **Semen pH**: Alkaline and ranges from 7.2–8 (>7.2). The pH should be measured after liquefaction, preferably after 30 minutes.

Microscopic Examination

Q. Write short note on microscopic examination of semen.

- **Sperm aggregation or agglutination**: The adherence either of immotile spermatozoa to each other or of motile spermatozoa to mucus strands, nonsperm cells or debris is considered to be nonspecific aggregation and should be noted. **Agglutination** refers to **motile spermatozoa sticking to each other**, head-to-head, tail-to-tail or in a mixed way. Any motile spermatozoa that stick to each other by their heads, tails or midpieces should be recorded.
- **Cellular elements other than spermatozoa**: During microscopic examination search should be made for the presence of cells other than spermatozoa. Some of these cells may be clinically relevant, which includes epithelial cells from the genitourinary tract and “round cells” (leukocytes and immature germ cells).
- **Assessment of sperm motility**: Motility of the sperms helps in penetration of cervical mucus and migration of the sperms into the fallopian tube. In normal semen, 38–42% of sperms should be motile (progressively motile and non-progressively motile). This is assessed by placing a drop of liquefied semen on a clean glass slide with a coverslip placed over it and examining under microscope. According to the WHO (2010), the **motility of each spermatozoon (plural is spermatozoa) is categorized as progressively motile (PR), nonprogressively motile (NP) and immotile (IM)**. This grading system has replaced the older grading system of a, b, c or d (I to IV) grades.
- **Sperm vitality**: It is important to know whether immotile spermatozoa are alive or dead. Normally, 55–63% live forms are observed. Vitality of the spermatozoa is estimated by identifying those with an intact cell membrane and is especially important for samples with less than about 40% progressively motile spermatozoa. The percentage

of live spermatozoa is assessed either by dye exclusion or by hypotonic swelling.

Q. Write short note on sperm count.

- **Total sperm count:** Sperm count is carried out in an improved Neubauer chamber using a Thoma pipette in a dilution of 1 in 20 (as for total leukocyte count) using semen diluting fluid. **Normal range of sperm count:** 33–46 millions/ejaculate.
 - **Aspermia:** No semen.
 - **Azoospermia:** No spermatozoa in the ejaculate.
 - **Oligospermia:** Total number of spermatozoa below the lower reference limit.
- **Sperm morphology:** Smear prepared from semen is fixed and stained with Papanicolaou stain to identify the morphological features. Normal spermatozoa have a head, neck, middle piece (midpiece), principal piece and endpiece. Since the endpiece is difficult to see under light microscope, the spermatozoa can be considered to consist of a head (and neck) and tail (midpiece and principal piece). Normally, more than 30% of sperms have normal morphology. For a spermatozoon to be considered as normal, both its head and tail must be morphologically normal. All other forms should be considered as abnormal.

Q. Write short answer on abnormal forms of sperm.

Classification of abnormal sperm morphology: Abnormal spermatozoa usually have a lower fertilizing capacity. The abnormalities in morphology can be classified as:

1. **Head defects**
 - **Abnormal size:** Large or small head.
 - **Abnormal shape:** Pointed/tapered, round, pyriform, amorphous.
 - **Acrosomal defects:** Vacuolated acrosomal region, abnormality of acrosomal areas.
 - **Abnormal number:** Double-headed.
2. **Neck and midpiece defects:**
 - Sharply bent neck.
 - Asymmetrical insertion of the midpiece into the head.
 - Thick or thin or irregular midpiece.
3. **Tail (principal) piece defects:** These abnormalities include short or bent or coiled or double tail.

Tests for Antibody Coating of Spermatozoa

The agglutination of spermatozoa may be the result of the anti-sperm antibodies (ASAs) or other factors present in the semen. ASAs in the semen belong to IgA and IgG types and it may also be found in sperm-free fluids (seminal plasma, blood serum and cervical mucus).

Tests for antibodies on spermatozoa (“direct tests”): Two direct tests include the mixed antiglobulin reaction (MAR) test and the immunobead (IB) test.

Tests for anti-sperm antibodies in sperm-free fluids (“indirect” tests).

SPUTUM EXAMINATION

Q. Write short essay/note on sputum examination and its importance.

Sputum is a highly specialized watery, colorless and odorless product of the respiratory tract. Expectored sputum is always abnormal and it consists of mucus and a variety of cellular and noncellular materials. It is the most frequently received specimen from the respiratory tract. Both its collection and examination are advantageous as samples are easily obtained, cost effective and its cellular content is representative of the entire respiratory tract.

Indications for Sputum Examination

Q. Write short answer on indications for sputum examination.

1. Smear and culture **identification of causative organisms** in suspected infection (e.g. pneumonia, tuberculosis).
2. Cytological examination for **malignant cells, viral inclusions, asbestosis**.

Sputum Collection

The patient is instructed to cough up to get the sputum proper and the same is collected in a wide-mouthed sterile, glass/plastic container with screw cap.

In those patients who cannot produce sputum spontaneously by deep coughing, a specimen of sputum may be induced. This is done by inhalation of appropriate solvents which are aerosolized to stimulate sputum production.

Early morning sputum sample is preferred for routine examination and 24-hours sample for the demonstration of tubercle bacilli by concentration method.

Examination of Sputum

Sputum examination consists of (i) physical examination, (ii) microscopic examination and (iii) culture study.

Physical Examination

- **Quantity**
 - In bronchiectasis, large amount of purulent sputum is coughed out.
 - Large amount of watery sputum with pink tinge suggests pulmonary edema.
- **Appearance/color:** Colors of sputum vary in different pathological conditions.
- **Odor or smell:** Foul smelling sputum is observed in bronchiectasis and lung abscess and is due to anaerobic bacterial infections.

Microscopic Examination

Staining of sputum: Two to three smears are made on a clean dry glass slides and are stained with:

- Leishman stain or Wright stain for differential count.
- Other stains (depends on the clinical/pathological features).
- Gram's stain for microorganisms.
- Ziehl–Neelsen stain for acid-fast tubercle bacilli.
- Special stains for fungi.
- Papanicolaou stain for study of malignant cells.

Cells

Normal sputum consists of a few neutrophils, few lymphocytes, carbon-laden macrophages, occasional eosinophils and red cells. Various types of cells seen in sputum and their significance are shown in Table 13.13.

BLOOD GROUP SYSTEM

Q. Write short essay/note on blood grouping.

More than 400 red blood cell antigens have been identified, most of which are inherited in a Mendelian dominant fashion and only few are clinically important. These antigens form more than 20 genetically determined blood group systems. The two most important blood group systems are ABO and Rh systems.

Q. Write short essay/note on methods of blood grouping.

ABO Blood Group System

Q. Write short essay/note on ABO blood group.

ABO Antigens

According to the ABO blood group system, there are four different kinds of blood groups: A, B, AB or O. The antigen on red cells may be A/B/AB or no antigen at all. There are two

TABLE 13.13: Types of cells in sputum and their significance

Type of cell	Significance
Pus cells (neutrophils)	Numerous pus cells indicate pyogenic infection of the respiratory tract
Eosinophils	Increased eosinophils are seen in asthma and parasitic infections of the lungs
Lymphocytes	Increased lymphocytes are seen in early pulmonary tuberculosis
Red blood cells	If increased, indicate hemorrhage (bleeding) into the lungs or the bronchi
Heart failure cells (hemosiderin-laden macrophages)	Chronic venous congestion of the lungs Pulmonary infarction and pulmonary hemorrhage
Anthraxotic pigment-laden cells (carbon-laden)	Increased in coal workers pneumoconiosis and those who live in smoky polluted atmosphere

subgroups in group A, namely A1 and A2. A and B antigens are inherited as per the Mendelian laws. Each individual inherits two ABO genes, one from each parent and these genes determine the ABO antigens on their red blood cells. Absence of both antigens, A and B on the red cells is seen in blood group O.

ABO Antibodies

Natural ABO Antibodies

The serum contains naturally occurring antibodies against red cell A/B/AB antigens. These are of IgM type and do not cross the placental barrier.

Acquired ABO Antibodies

An individual lacking a particular antigen may develop an antibody after (acquired) exposure to red blood cells carrying the corresponding antigen. Exposure occurs due to parenteral introduction of red cell antigens. This may develop during:

- **Transfusion of red cells:** These acquired antibodies cause hemolytic transfusion reaction.
- **Pregnancy:** Passage of fetal red cells (having paternal antigens foreign to the mother) into the maternal circulation during pregnancy may result in mainly IgG type of antibodies. They can cross the placental barrier resulting in hemolytic disease of the newborn. The most important acquired *antibody* is anti-D, which is a major cause of hemolytic disease of the newborn.

H Genes and H Antigens

The expression of A and B antigens are dependent on the presence of H gene. Most of the individuals are homozygous for the H gene. The sequence of events in the formation of A and B red cell antigens is:

- A and B red cell antigens are glycoproteins and their formation starts with basic precursor substance.
- Basic precursor substance is first converted into H substance (by transferase) under the influence of H gene.
- H substance is partially converted under the influence of A and B genes (and specific transferase) into A and B antigens. Some of the H substance remains unconverted.
- Since, O group individuals do not have A and B genes, neither A nor B antigen is formed and these have only H substance (H substance remains unchanged).

Note: A, B and H antigens may be detected in the saliva and other body fluids also. Such individuals are called **secretors**, while the remaining without it are **nonsecretors**.

Bombay Blood Group

- In 1952, Bhende, Bhatia and Deshpande discovered a new blood group known as Bombay blood group (phenotype Oh). These individuals lack the H gene and therefore, the basic precursor substance cannot be converted into H substance. This in turn results in failure to form A or B antigen.
- When their blood sample is tested for routine ABO grouping, they will be labelled as blood group O. However, their serum contains anti-A, anti-B and anti-H antibodies. These individuals therefore, should be transfused with only Bombay blood group.

ABO Grouping Technique

- ABO grouping is of two types namely (i) forward (cell) typing in which antigens on RBCs are detected and (ii) reverse (serum) typing in which antibodies in the serum/plasma is detected.
- ABO grouping is performed by making a 2% saline suspension of red cells and adding anti-A, anti-B and anti-AB sera.

Methods: The different methods available are as follows: Slide or tile technique, tube technique, microplate method, microtyping system and automated or semi-automated method.

Slide or Tile Technique

- Take a slide/white tile and mark it anti-A, anti-B and anti-AB. Put one drop each of anti-A, anti-B and anti-AB sera on the marked slide. Add one drop of washed

2% red cell suspension to each anti-sera. Mix each one separately with clean applicator sticks and spread the mixture over an area of 2 cm. Rock the slide gently and look for "agglutination" within 5 minutes.

- **Interpretation:** Blood group A, B, AB or O is interpreted depending on the agglutination in the antisera.
- **Controls:** Each blood group test should preferably have controls, both positive and negative.
- **Positive control:** This is run by performing the above techniques by using cells of known groups A, B, AB and O. This is especially useful for testing the potency of antisera.
- **Negative control:** Run the above tests by adding saline instead of antisera. If agglutination develops, it indicates autoagglutination or pseudoagglutination.

Note:

- A group has two subgroups namely A1 and A2, which can be differentiated by using anti-A1 lectin.
- Presence of H substance can be detected by anti-H.

Rh Blood Group System

Q. Write short essay/note on Rh factor and Rh blood grouping.

Rh blood group system is the second system of clinical significance in transfusion medicine. This is a complete system of antigens which are labeled as D, d, C, c, E and e. The D antigen is highly immunogenic. Cc and Ee antigens are weak antigens and therefore, risk of sensitization to these antigens is less than the risk of sensitization to D.

Rh (D) System

Rh Antigen

- **Rh positive (+ve):** Individuals who have D antigen on the red cell surface are Rh positive (+). This may be present as homozygous (DD) or heterozygous (Dd) state.
- **Rh negative (-ve):** Individuals who lack the D antigens are called as Rh-negative.

Rh Antibodies

- In contrast to ABO system, there are no naturally occurring antibodies against Rh antigens in Rh negative individuals.
- Acquired (immune) antibodies: Rh antibodies develop after exposure to Rh antigens either following transfusion (Rh +ve blood given to Rh -ve patients) or bind complement. They can be detected by antiglobulin test (Coombs test).

Clinical Significance

- **Rh incompatibility between donor and recipient** results in hemolytic transfusion reactions.

- **Rh incompatibility between mother and fetus** results in **hemolytic disease of the newborn (HDN)**.

Rh (D) Typing Techniques

Methods: The different methods available are: Slide or tile technique, tube technique, microplate method, microtyping system and automated or semi-automated method.

Slide or Tile Techniques

- Place one drop of anti-Rh (D) reagent (should be monoclonal IgM type) on a slide/white tile. Add 1 drop of 2% red cell suspension. Mix them using a clean applicator stick. Observe for agglutination after 2 minutes.
- **Interpretation:** Presence of agglutination indicates that the blood sample is Rh +ve.
- **Controls:** Known Rh +ve and Rh -ve samples should be run as positive and negative control.

Importance of Blood Group

Q. Write short answer on importance of blood group.

Blood grouping is important before blood transfusion and is also of medicolegal value.

TRANSFUSION MEDICINE

Transfusion medicine comprises of blood and blood component transfusion. Blood cannot be synthesized artificially. So, the source of blood is from a healthy human donor.

Blood Transfusion

Blood transfusion is the process of transferring blood/ blood products from donor into the circulating system of recipient. It is important to properly collect the blood from donor, prepare its components (if required) and store blood/ components in a proper way and transfuse in such a way to avoid any risks or hazards.

Donor Selection

Q. Criteria for selection of blood donor.

Donor selection is based on medical history and few routine physical examinations (weight, blood pressure, temperature, hemoglobin) are done to know whether donor is suitable for donating blood.

- Donor should be healthy. There are three types of donors namely voluntary (should be encouraged), replacement and professional.

- It is important to know whether the patient has history of diseases like hepatitis, AIDS, syphilis and if so, blood should not be obtained from them.

Collection of Blood

Blood is collected under aseptic conditions using sterile, plastic bag with anticoagulant. Now CPD-A is used as an anticoagulant. Mix the blood and anticoagulant gently and periodically during its collection.

Q. Write short answer on anticoagulants used in blood bank.

Anticoagulants used: The different anticoagulants-preservative solutions available are as follows:

- Citrate phosphate dextrose (CPD).
- Citrate phosphate dextrose adenine (CPDA-1): Functions of various chemical used is mentioned in Table 13.23.
- Acid citrate dextrose (ACD) is not used nowadays.
- Functions of component of CPDA-1 (Table 13.14).

Q. Write short answer on functions of each component of CPDA-1.

Storage of blood: Blood is stored in a refrigerator at 2°–6°C.

Predonation Check-Up

Q. Write short answer on screening of blood donor.

Donor blood: The following tests are routinely carried out on donor's blood.

- ABO and Rh grouping.
- Tests for:
 - HBsAg, anti-HCV, anti HIV-1 and HIV-2 and serum alanine aminotransferase (ALT).
 - Malaria and syphilis.

Recipient blood: The recipient's ABO and Rh grouping is also carried out.

Compatibility Testing (Pretransfusion Testing)

Before transfusion of any blood or its components, it is essential to know whether they are compatible with the recipient's blood. This is achieved by performing a set of

TABLE 13.14: Functions of various chemicals used in anticoagulant- preservative solution

Chemical used	Function
Citrate	Prevents clotting by chelating calcium
Sodium biphosphate	Buffers the end product of glycolysis (lactic acid)
Dextrose	Needed for ATP generation for viability of red cells
Adenine	Substrate for red cell synthesis of ATP

procedures known as compatibility testing. Sometimes the term compatibility test and cross matching are used interchangeably, but cross-match is only a part of compatibility test. Compatibility tests include:

- Review of patient's past blood bank history and records (if done earlier).
- ABO and Rh typing of the recipient and donor.
- Antibody screening test of recipient's and donor's serum.
- Cross-matching.

Cross-matching

Q. Write short answer on cross-matching.

- Cross-matching is very important before any blood transfusion. Cross-matching should be carried out to ensure that there are no antibodies in patient's serum that will react with the donor's cells when transfused.

Importance of Cross-matching

- It is the final check of ABO compatibility between donor and recipient.
- Detects the presence of any clinically significant, unexpected antibodies in the recipient's serum that may react with donor's cells, thereby preventing any transfusion reaction.
- A thorough cross-matching is performed with recipient's serum and donor's red cells.

Types

Cross-matching procedure may be divided into two major parts, namely major and minor cross-matching (Tables 13.15)

It is always necessary to monitor the recipient during and after transfusion so that any complications can be dealt accordingly.

Blood Components

Q. Write short essay/note on blood components and fresh frozen plasma.

It is possible to separate different components of blood from a single unit of whole blood. These components can be

TABLE 13.15: Types of cross-match

Type of cross-match	Donor's	Recipient's (patient's)
Major cross-match	Red cells	Serum
Minor cross-match	Serum	Red cells

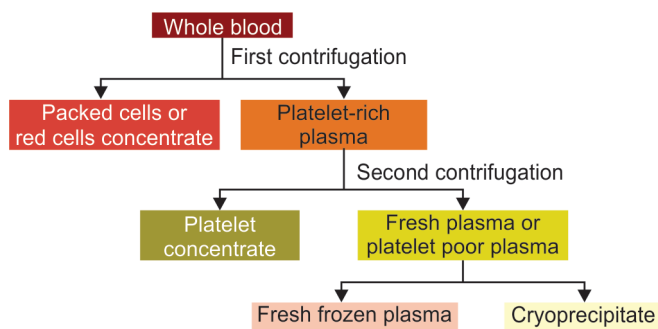


Fig. 13.6: Blood components

used individually to help more than one patient with many purposes. Thus, red cells can be transfused to an anemic patient and plasma for a burns patient. This also ensures that only the required components are transfused. The various blood components (Fig. 13.6) are as follows:

- Red cells concentrate or packed red blood cells (PRBCs).
- Platelet concentrates.
- Plasma.
- Fresh frozen plasma (FFP).
- Cryoprecipitate.

Platelet Concentrate

Q. Write short answer on platelet concentrate.

Platelet concentrate may be obtained from a single donor or pooled plasma. Platelets can also be obtained from a single donor by platelet apheresis. Indications for platelet concentrate transfusion are listed in Box 13.17.

BOX 13.17: Indications for platelet concentrate transfusion

Bleeding due to:

- Severe thrombocytopenia (when platelet count is less than 20,000/cu mm)
 - Immune mediated: In patients with autoimmune thrombocytopenia, it should be reserved for patients with life threatening bleeding
 - Secondary to bone marrow failure
 - ◆ Chemotherapy induced
 - ◆ Due to leukemia
 - ◆ Dilutional
- Abnormal platelet function
- Disseminated intravascular coagulation (DIC)
- Surgical or invasive procedures in thrombocytopenic patients

Fresh Frozen Plasma (FFP)

Q. Write short answer on fresh frozen plasma.

Fresh frozen plasma (FFP) is prepared by freezing the plasma and contains plasma proteins and all coagulation factors that include albumin, protein C and S, antithrombin and von Willebrand factor. It is indicated for replacement of coagulation factors in acquired coagulation factor deficiencies (Box 13.15).

BOX 13.15: Indications for fresh frozen plasma

- Patients on anticoagulant drug therapy (Coumarin)
- Antithrombin deficiency
- Coagulopathy of liver diseases
- Vitamin K deficiency
- Microangiopathic hemolytic anemia including TTP, hemolytic uremic syndrome and HELLP syndrome
- DIC

Transfusion Reactions

Q. Write short answer on transfusion reactions.

Blood transfusion is useful and lifesaving when performed with caution and with clear indication. Sometimes (about 2–4% of cases) unfavorable complications occur inspite of precaution and preventive measures, which are known as transfusion reactions. They may be broadly divided into infectious and noninfectious complications (Box 13.18).

BOX 13.18: Complications of blood transfusion

Noninfectious complications

- **Immediate reactions**
 - Immunological: Hemolytic transfusion reactions, febrile non-hemolytic reaction, allergic reaction, anaphylactic, transfusion-related acute lung injury (TRALI)
 - Non-immunological: Circulatory overload, air embolism
- **Delayed reactions**
 - Immunological: Alloimmunization, hemolytic transfusion reactions (mostly asymptomatic), transfusion-associated graft versus host disease
 - Non-immunological: Iron overload-transfusion hemosiderosis, thrombophlebitis

Infectious complications: Few of the diseases transmitted by transfusion are as follows:

- Hepatitis (HBV, HCV and HDV)
- HIV (AIDS)
- Malaria
- Cytomegalovirus
- Syphilis

BOX 13.19: Liver function tests

A. Tests for excretory functions

- Bilirubin metabolism:
 - Serum bilirubin estimation
 - Bilirubin in feces
 - Bilirubin and urobilinogen in urine
- Bromosulphthalein (BSP) excretion
- Bile acids (bile salts)

B. Tests of liver cell injury

- Enzymes that detect hepatocellular injury
 - Transaminases (aminotransferases): Serum aspartate transaminases (AST), serum alanine transaminases (ALT), AST/ALT ratio
 - Lactate dehydrogenase (LDH)
- Enzymes that detect cholestasis
 - Serum alkaline phosphatase
 - γ -glutamyl transpeptidase
 - 5'-Nucleotidase

C. Tests of metabolism and biosynthesis

- Proteins secreted into the blood
 - Serum proteins: Albumin, globulin and albumin: globulin (A:G) ratio
 - Clotting factors: Prothrombin time
- Hepatocyte metabolism: Serum ammonia
- Lipid and lipoprotein metabolism
- Carbohydrate metabolism

D. Immunological tests

E. Ancillary diagnostic test: Alpha-fetoprotein (AFP)

LIVER FUNCTION TESTS

Q. Name liver function tests.

Liver function tests (LFTs) are groups of laboratory tests designed to give information about the state of a patient's liver (Box 13.19). Because of multiple and complex functions of the liver, it is necessary to perform several tests for an accurate diagnosis, to assess the severity of the disease and its prognosis.

Liver Biopsy

Percutaneous needle biopsy of the liver through an intercostal approach is done under local anesthesia. It is a simple, safe procedure for assessment of liver histology for the diagnosis of various liver disorders.

Before the biopsy: Prothrombin time (PT) should be performed before liver biopsy. If it is high, patient should be given parenteral vitamin K and test should be repeated. If PT is prolonged by more than 3 seconds than the control, it is a contraindication for liver biopsy.

TABLE 13.16: Thyroid function tests

Test	Significance
Serum thyroid hormones (normal values)	
• Thyroid stimulating hormone/thyrotrophin (TSH) 0.4–5.0 µU/mL	Increased in primary hypothyroidism, Hashimoto thyroiditis. Decreased in hyperthyroidism
• Thyroid hormone levels: Free thyroxine (FT4) (5.4–11.7 µg/dL), triiodothyronine (T3) (77–135 ng/dL)	Increased in hyperthyroidism and decreased in hypothyroidism
• Thyrotrophin releasing hormone (TRH) stimulation test (0.4–5.0 µU/mL)	In primary hypothyroidism an exaggerated prolonged rise of already increased TSH level is observed. In secondary hyperthyroidism (pituitary), there will be no rise in the decreased TSH level
• Thyroglobulin (Tg)	Increased in well-differentiated thyroid carcinoma and hyperthyroidism. Decreased following total thyroidectomy or destruction of thyroid by radiation
• Calcitonin level	Used for diagnosis of primary/recurrence of medullary carcinoma of thyroid or metastases after the removal of the primary. Increased in some paraneoplastic syndromes (e.g. carcinoma of lung), hypercalcemia of any cause and C cell hyperplasia
Thyroid autoantibody tests	
• Anti-microsomal antibody • Anti-thyroid peroxidase antibody (TPOAb) • Anti-thyroglobulin antibody (TgAb) • TSH receptor antibody (TRAb)	Diagnosis and monitoring of autoimmune thyroid diseases, Hashimoto thyroiditis: Antimicrosomal or antithyroid peroxidase antibodies, Graves' disease: Anti-TSH receptor antibodies
Radioactive iodine uptake (RAIU)	Increased in Graves' disease, toxic multinodular goiter and adenoma and early thyroiditis. Decreased in hypothyroidism, late thyroiditis

Needle: Vim Silverman's needle is used for liver biopsy.

Indications for Liver Biopsy

Q. Indications for liver biopsy.

- Unexplained hepatomegaly, splenomegaly, jaundice.
- Cirrhosis of liver, to assess the type, etiology and activity of the disease process.
- Chronic hepatitis: For identifying, grading of inflammatory activity, staging and evaluation of the course.
- Pyrexia of unknown origin (PUO): A liver biopsy may be helpful in the diagnosis of miliary tuberculosis, sarcoidosis or malignancy.
- Idiopathic hemochromatosis: To determine iron stores in liver.

Contraindications

Q. Contraindications for liver biopsy.

- Congenital coagulation disorders, e.g. hemophilia A and B.
- PT prolonged by more than 3 seconds over control.

- Obstructive jaundice: Liver biopsy may cause leakage of bile from liver into the peritoneum causing severe and fatal bile peritonitis.
 - Massive ascites.
 - Patients with severe cough where diaphragm movements cannot be controlled.
 - Uncooperative patients.
 - Hydatid cyst liver
 - Hemangioma liver
- } These are relative contradictions.

Complications

- Hemorrhage from the biopsy site.
- Bile peritonitis in patients with obstructive jaundice.
- Referred pain to shoulder from right pleura.

RENAL FUNCTION TESTS

Q. Name renal function tests.

- Urine analysis (both routine and microscopic).

- Blood chemistry: Impaired renal function causes elevation of end-products of protein metabolism which is known as **azotemia**. These include:
 - Blood urea (normal range 20–40 mg/dL).
 - Blood urea nitrogen (BUN): Normal range 10–20 mg/dL.
 - Creatinine (normal range 0.6–1.2 mg/dL).
- Renal clearance tests.
- Concentration and dilution tests.

THYROID FUNCTION TESTS

Q. Write short essay on thyroid function tests.

Thyroid function tests (TFTs) is a collective term for test used to check the function of the thyroid gland. Various tests and their significance are mentioned in Table 13.16.

3

SECTION

Systemic Pathology

- | | |
|--|--------------------------------------|
| 14. Vascular Disorders | 22. Male Genital Tract Disorders |
| 15. Heart Disorders | 23. Female Genital Tract Disorders |
| 16. Lung Disorders | 24. Breast Disorders |
| 17. Oral Cavity and Salivary Gland Disorders | 25. Endocrine Disorders |
| 18. Gastrointestinal Tract Disorders | 26. Skin Disorders |
| 19. Hepatobiliary Disorders | 27. Bone and Joint Disorders |
| 20. Pancreatic Disorders | 28. Central Nervous System Disorders |
| 21. Kidney and Urinary Tract Disorders | |

Vascular Disorders

ARTERIOSCLEROSIS

Arteriosclerosis (“hardening of the arteries”) is characterized by arterial wall thickening and may be caused by:

1. **Arteriolosclerosis:** It affects small arteries and arterioles.
2. **Mönckeberg medial sclerosis:** It is characterized by deposition of calcium in muscular arteries seen in old age (above 50 years).
3. **Atherosclerosis:** It is the most frequent and important disease of intima.

Capillaries: Maximum cross-sectional surface area.

Maximum blood volume present in the veins.

ATHEROSCLEROSIS

Atherosclerosis: Primarily a disease of intima characterized by lesions called atheroma.

Definition: Atherosclerosis is primarily a progressive **disease of intima involving large and medium-sized elastic and muscular arteries**. It is characterized by **focal lipid-rich intimal lesions called atheromas** (atheromatous or atherosclerotic plaques).

Epidemiology: Atherosclerosis is a worldwide disease seen in both developed and developing countries.

The word atherosclerosis is derived from Greek for “gruel” (atheroma) and “hardening” (sclerosis).

Risk Factors for Atherosclerosis

- They were identified through several studies most important being the Framingham Heart Study and

Atherosclerosis Risk in Communities Study. Risk roughly increases with the increase in number of risk factors. For example, two factors increase risk about four-fold, and three (i.e. hyperlipidemia, hypertension, and smoking), increase risk by a factor of seven.

- **Classification of risk factors:** The risk factors may be broadly classified as modifiable, nonmodifiable and additional (Table 14.1).

Atherosclerosis: Major modifiable risk factors include hyperlipidemia, hypertension, cigarette smoking and diabetes.

Modifiable Risk Factors in Ischemic Heart Disease (IHD)

These are **four** in number namely: (1) hyperlipidemia, (2) hypertension, (3) cigarette smoking, and (4) diabetes.

1. **Hyperlipidemia: Increase in the serum lipids** mainly cholesterol (**hypercholesterolemia**) is a major modifiable

Q. Write short note on risk factors for atherosclerosis.

TABLE 14.1: Risk factors for atherosclerosis

A. Modifiable major risk factors	
• Hyperlipidemia	• Cigarette smoking
• Hypertension	• Diabetes mellitus
B. Nonmodifiable (constitutional) risk factors	
• Genetic abnormalities	• Increasing age
• Family history	• Male gender
C. Additional risk factors	
• Inflammation	• Raised procoagulant levels
• CRP level	• Inadequate physical activity
• Hyperhomocystinemia	• Stressful lifestyle
• Metabolic syndrome	• Obesity
• Lipoprotein (a)	• Alcohol

risk factor. Hypercholesterolemia can initiate atherosclerotic lesion even in the absence of other risk factors. The major components of lipids in the blood are:

- **Low-density lipoprotein (LDL)** known as “**bad cholesterol**” is one of the components of serum lipid and its **higher level** is associated with **increased risk of atherosclerosis**. LDL delivers cholesterol to peripheral tissues.
- **High-density lipoprotein (HDL)** known as “**good cholesterol**” and its **higher levels** are associated with **decreased risk**.
 - HDL mobilizes cholesterol from the periphery (including atheroma) and excretes it through bile in liver.
 - Exercise and moderate consumption of ethanol raise HDL levels, whereas obesity and smoking lower it.
- **Serum cholesterol level:** Normal range is 140–240 mg/dL. Serum cholesterol is **strongly related to the dietary intake of saturated fat** (in the absence of genetic disorders of lipid metabolism). Risk of atherosclerosis increases with increasing serum cholesterol concentrations and lowering serum cholesterol concentrations reduces the risk. The risk of atherosclerosis can be reduced by lowering LDL or total plasma cholesterol, and/or raising serum HDL. This can be achieved either by dietary modification or by treatment with cholesterol-lowering drugs.
 - **Diet which raises blood cholesterol:**
 - ♦ **Diet high in cholesterol and saturated fats** (e.g. egg yolks, animal fats, and butter). Transunsaturated fats produced by artificial hydrogenation of polyunsaturated oils (used in baked goods and margarine).
 - ♦ **Diet which lower blood cholesterol:** Diets low in cholesterol and/or with higher ratios of polyunsaturated fats.
 - ♦ **Omega-3 fatty acids** (high in fish and fish oils).
 - **Cholesterol-lowering drug:** For example, statins lowers circulating cholesterol levels by inhibiting hydroxymethylglutaryl coenzyme A (HMG CoA) reductase. This is the rate-limiting enzyme in involved in cholesterol biosynthesis in the liver.

LDL: Bad cholesterol; HDL: Good cholesterol.

HDL: Good cholesterol removes cholesterol from atheromatous plaque and transports to liver. From the liver, it is excreted in the bile.

Disorders associated with hypercholesterolemia

1. Nephrotic syndrome
2. Alcoholism
3. Hypothyroidism
4. Diabetes mellitus.

HDL: Removes cholesterol from atheromatous plaque for disposal in the liver.

Increased very low density lipoprotein (VLDL) leads to reduced HDL.

2. **Hypertension:** It is one of the major risk factor.
 - Incidence of atherosclerosis increases as BP rises, and this excess risk is **related to both systolic and diastolic levels of blood pressure**.
 - Control of hypertension with antihypertensive therapy reduces the risk of myocardial infarction and stroke.
3. **Cigarette smoking:** It is the **most important avoidable cause** of atherosclerosis.
 - Atherosclerosis is **more severe and extensive among smokers than in nonsmokers**. This may be the reason for the increased incidence and severity of atherosclerosis in men compared to women. **There is a strong dose-linked relationship between cigarette smoking and ischemic heart disease. Cessation of smoking reduces the risk.**
4. **Diabetes mellitus:** It is a potent risk factor for atherosclerosis.
 - Diabetes is associated with hypercholesterolemia → increases the risk of atherosclerosis. The incidence of myocardial infarction and other atherosclerotic vascular diseases (strokes, gangrene of the lower extremities) is more in diabetics than in nondiabetics.

Nonmodifiable/Constitutional Risk Factors

1. **Genetic abnormalities:** Most common inherited modifiable risk factors (hypertension, hyperlipidemia, and diabetes mellitus) are polygenic. Mendelian disorders, such as **familial hypercholesterolemia** are associated with atherosclerosis.
2. **Family history:** Atherosclerotic disease often runs in families. Familial predisposition is usually multifactorial, due to genetic, environmental and lifestyle factors.
3. **Increasing age:** Age is the most powerful independent risk factor. Clinical manifestation of atherosclerosis is usually observed after middle age and the lesions progressively rise with each decade.
4. **Sex: Premenopausal women have lower incidence** of atherosclerosis-related diseases compared to males of the same age group. However, after menopause this sex difference disappears. This may be due to protective role of estrogen. However, hormone replacement therapy has no role in the prevention of coronary heart disease.

Achilles tendon xanthoma: Pathognomonic of familial hypercholesterolemia.

Additional Risk Factors

1. **Inflammation:** It plays a role in atherogenesis and may be a risk factor.
2. **C-reactive protein (CRP) level:** It is a marker of systemic inflammation and **predicts the risk** of atherosclerosis related diseases.
3. **Hyperhomocysteinemia:** It is a rare autosomal recessive inborn error and results in elevated circulating homocysteine → premature and severe atherosclerosis.
4. **Metabolic syndrome:** It is associated with central obesity, insulin resistance and is known risk factors for atherosclerosis.
5. **Increased Lipoprotein (a) Lp(a) levels:** It is an altered form of LDL and is associated with increased risk. Lp(a) has structural similarity to plasminogen. It competes with plasminogen in clots and decreases the ability to form and clear clots. Increased Lp(a) levels are associated with higher risk of atherosclerosis, independent of total cholesterol or LDL levels.
6. **Raised procoagulant levels:** These procoagulants include, thrombin (procoagulant and proinflammatory action), platelet activation and raised fibrinogen → increased risk.
7. **Inadequate physical activity:** Lack of exercise doubles the risk.
8. **Stressful lifestyle:** Certain personality associated with competitive, stressful life ("type A" personality) is associated with an increased risk of coronary disease.
9. **Obesity:** It is often associated with hypertension, diabetes mellitus, hypertriglyceridemia, decreased HDL and physical inactivity.
10. **Alcohol consumption:** It is associated with reduced rates of coronary artery disease.

C-reactive protein (CRP) level.

C-reactive protein: Excellent marker for disrupted atheromatous plaque.

Regular exercise (brisk walking, cycling or swimming for 20 minutes two or three times a week) has a protective effect.

Excess alcohol consumption is associated with hypertension and cerebrovascular disease.

Pathogenesis of Atherosclerosis

Q. Write short note on pathogenesis of atherosclerosis.

There are several theories proposed for pathogenesis of atherosclerosis and few of them are:

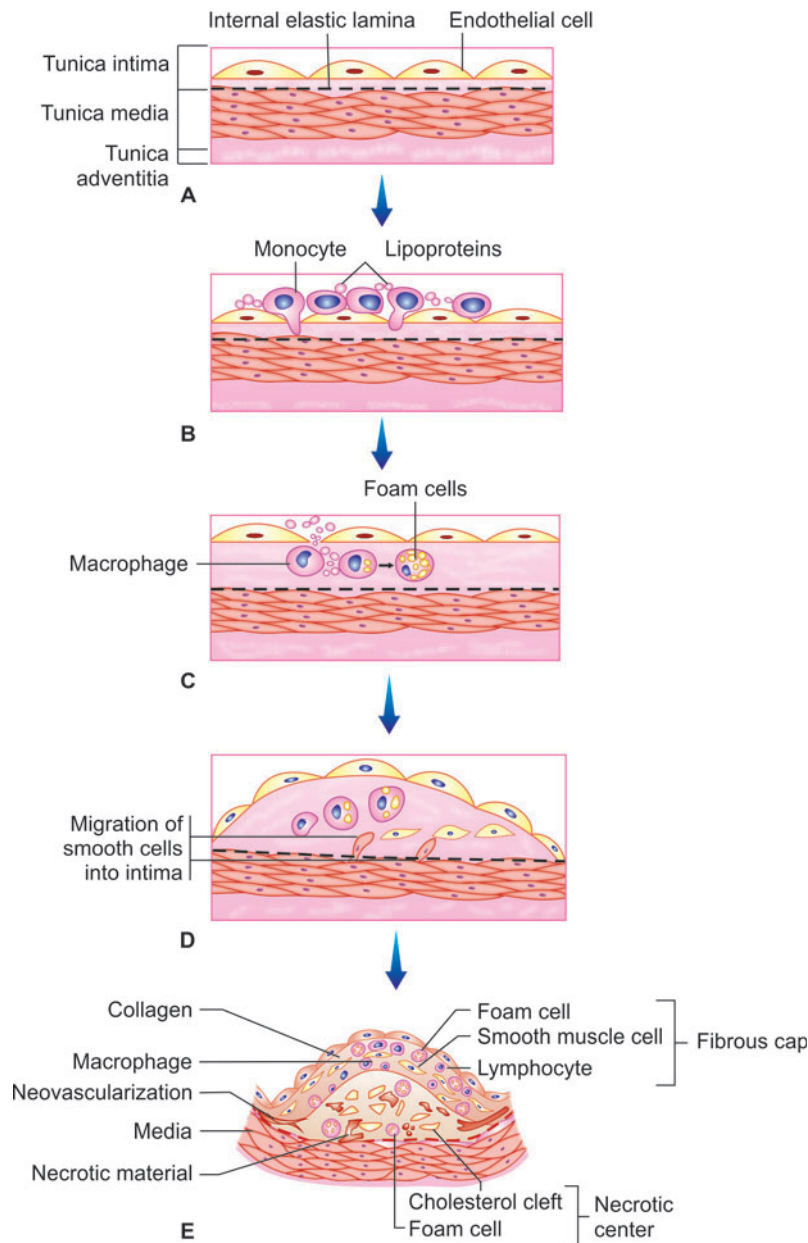
- A. Insudation hypothesis:** According to this, the focal accumulation of lipid in a vessel wall is due to insudation (transport) of plasma lipoproteins across an intact endothelium.
- B. Encrustation hypothesis:** This hypothesis states that small mural thrombi are formed at the site of endothelial damage. These thrombi become organized and form plaque.
- C. Monoclonal hypothesis:** The monoclonal concept postulates that single clone (monoclonal) of smooth muscle migrate from the underlying media into the intima and then proliferate. The stimulus for monoclonal proliferation may be metabolites of cholesterol.
- D. Response-to-injury hypothesis:** This is the currently favored hypothesis.

Response-to-Injury Hypothesis (Figs 14.1A to E)

Q. Write short note on reaction-to-injury hypothesis of atherosclerosis.

According to this theory, **atherosclerosis develops as a chronic (inflammatory and healing) response of the arterial wall to the endothelial injury**. Probably accumulation of cholesterol crystals and free fatty acids in macrophages and other cells initiate inflammation. It is thought that chronic inflammation is responsible for both the initiation and progression of atherosclerotic lesions. The **sequence of pathogenic events** (Figs 14.1A to E) is as follows:

1. **Endothelial injury and dysfunction:**
 - **Causes of endothelial injury/dysfunction:**
 - **Hemodynamic disturbances:** Plaques develop in regions having disturbed blood flow such as origin or ostia of vessels, branching points of vessel and along the posterior wall of the abdominal aorta.
 - **Risk factors:** Hyperlipidemia, hypertension, toxins from cigarette smoke, and advanced glycation end products in diabetes can produce endothelial injury/dysfunction.
 - **Others:** Homocysteine, immunocomplexes, and infectious agents.
 - **Effect of endothelial injury or dysfunction:**
 - **Leukocyte (mainly monocyte) adhesion** to endothelium
 - **Increased vascular permeability**
 - **Platelet adhesion and thrombosis**
 - **Movement of low-density lipoproteins (LDLs) across the endothelium into the intima.**
2. **Migration of monocytes into the intima:**
 - The **leukocytes**, which adhere at the site of endothelial injury/dysfunction, are **mainly monocytes and**



Figs 14.1A to E: Various steps in response to injury hypothesis of atherogenesis. (A) Normal; (B) Endothelial injury/dysfunction with adhesion of monocytes; (C) Migration of monocytes into the arterial intima and are transformed into macrophages. They engulf lipoproteins and oxidized LDL to form foam cells; (D) Migration of smooth muscle followed by their proliferation in the intima; (E) Fully developed atheromatous plaque

Foam cells in atherosclerosis: Lipid laden

1. Smooth muscle cells
2. Tissue macrophages
3. Blood monocytes.

Pathogenesis of atherosclerosis: Presently favored theory is reaction to injury hypothesis.

Infection: Herpes virus, *cytomegalovirus*, and *Chlamydia pneumoniae* have been found in atheromatous plaques. These infections may be responsible for local inflammatory reaction.

Risk factors in atherosclerosis: Cause endothelial injury/dysfunction and stimulate smooth muscle recruitment into the intima.

T lymphocytes. Accumulation of leukocytes initiates the atheroma formation.

- Locally produced chemokines allows penetration of the endothelial layer by monocytes and lymphocytes. The monocytes migrate into the intima where they are transformed into macrophages.

3. Lipid accumulation in the intima:

- Endothelial dysfunction also allows penetration of endothelium by lipoproteins (mainly LDL) from blood → LDLs accumulate within the intima of the vessel.
- LDL is oxidized by the action of oxygen free radicals produced locally by monocytes/macrophages and dysfunctional endothelial cells.

4. Formation of foam cells and activation of macrophages:

- **Macrophage engulfs lipoproteins and oxidized LDL** from the extracellular space in the intima. Their cytoplasm becomes foamy and these cells are called as **foam cells**.
- Some **foam cells may undergo apoptosis** → **release lipids** → form lipid-rich center, often called the necrotic core in atheromatous plaques.
- **Oxidized LDL is cytotoxic to endothelial cells and smooth muscle cells** and can cause endothelial dysfunction.
- **Oxidized LDL causes activated macrophages which produce:**
 - **Cytokine** (e.g. TNF)—**increases leukocyte adhesion**.
 - **Chemokines** (e.g. monocyte chemotactic protein 1)—**accumulation of monocytes**.
 - **Reactive oxygen species**—**aggravate oxidation of LDL**.
 - **Growth factors**—**stimulate smooth muscle cell proliferation and extracellular matrix (ECM) synthesis**.

5. Migration of smooth muscle cells into the intima:

- Growth factor (e.g. platelet-derived growth factor) from activated platelets, macrophages, and endothelial cells causes migration of smooth muscle cells **either from the arterial media or from circulating precursors**.

6. Smooth muscle cell proliferation in the intima and ECM production:

- Smooth muscle cells proliferate, and produce ECM (mainly collagen and proteoglycans). Many **growth factors can cause smooth muscle cell proliferation**. These includes: PDGF, fibroblast growth factor (FGF) and transforming growth factor- α (TGF- α).
- Smooth muscle cell **may also engulf oxidized LDL and form foam cells**.

7. Lipid accumulation

- Occurs both **intracellularly** (within macrophages and smooth muscle cells) and **extracellularly**.
- **Extracellular lipid** is derived from **insudation** from the vessel lumen (mainly in the presence of hypercholesterolemia) and also from **necrotic foam cells**.
- Cholesterol in the plaque is also due to an **imbalance between influx and efflux**. High-density lipoprotein (HDL) probably transfers cholesterol from these lesions and leads to its excretion by liver.

MORPHOLOGY

Q. Write short note on fatty streak.

Fatty Streaks: They may be the earliest or precursor lesions of atherosclerosis. They are found in young children (older than 10 years) as well as in adults.

- **Gross:** Multiple small (~1 mm) flat yellow lesions in the intima, which may coalesce to form long streaks 1 cm or more in length. They may be mildly elevated above intima without disturbing the blood flow.
- **Microscopy:** Consist of **lipid-filled foamy macrophages** in the intima.

Fatty streaks may be the earliest or precursor lesions of atherosclerosis and may be seen in children.

Atherosclerotic Plaque

Q. Differences between fatty streak and atherosclerotic plaque.

Q. Write short note on gross and microscopic features of atherosclerosis.

Q. Write short note on aortic lesions in atherosclerosis.

Gross (Fig. 14.2):

- **Sites:** Major vessels involved in atherosclerosis in descending order are:
 - **Lower abdominal aorta** (abdominal aorta more than the thoracic aorta)
 - **Coronary arteries**
 - **Popliteal arteries**
 - **Internal carotid arteries**
 - **Vessels of the circle of Willis.**
- **Color:** White to yellow. If superimposed by a thrombus → red-brown.
- **Size:** Range from 0.3 to 1.5 cm in diameter. Can coalesce to form larger masses. **Advanced lesions** are oval and range from 8 to 12 cm in diameter.
- **Shape:** Irregular with well-defined borders.
- **Distribution:** Patchy (focal) and usually involve only a portion of the involved arterial wall. On cross-section → appears as an eccentric lesion.
- **Composition:** Soft, yellow, grumous core of lipid covered by a white fibrous cap.

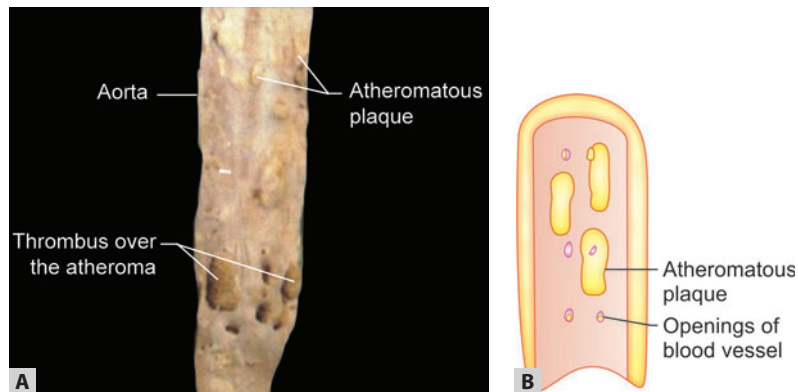
Atherosclerosis: Most common site is abdominal aorta.

Atherosclerosis: Microscopy shows necrotic center covered by fibrous cap.

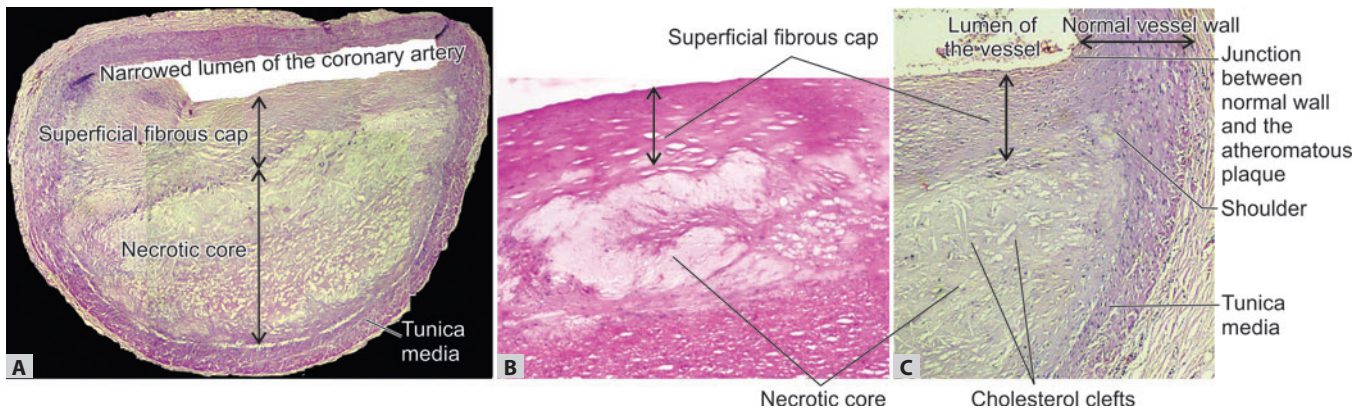
Microscopy (Figs 14.3 and 9.1E)

Three main components in varying proportions in different lesions:

1. **Cells:** Smooth muscle cells, macrophages, and T-cells.
2. **Extracellular matrix (ECM):** Collagen, elastic fibers, and proteoglycans.
3. **Lipid:** Both intracellular and extracellular lipid.



Figs 14.2A and B: (A) Atheromatous plaques on the intimal surface of aorta. Lower region shows thrombus over the plaque; (B) Diagrammatic gross appearance of atherosclerosis



Figs 14.3A to C: Microscopy of atherosclerotic plaque. (A) Scanner view of coronary atherosclerosis causing more than 90% narrowing of the lumen; (B) Shows central necrotic core and superficial fibrous cap; (C) Shows junction between the vessel wall and atheromatous plaque, shoulder region and cholesterol clefts in the necrotic core

These components occur in three regions:

- **Superficial fibrous cap:** Composed of **smooth muscle cells and collagen**.
- **Necrotic core:** It is seen deep to the fibrous cap and contains:
 - Lipid: Typical atheroma contains **abundant lipid, mainly cholesterol and cholesterol esters**, which is seen as empty needle-shaped cleft-like spaces.
 - Debris from dead cells.
 - **Foam cells** (lipid-laden macrophages and smooth muscle cells).
 - Others: Fibrin, organized thrombus, and plasma proteins.
- **Shoulder:** It is the peripheral region beneath and to the side of the cap. It is more cellular and contains macrophages, smooth muscle cells, and T-cells.

Neovascularization (proliferating small blood vessels): It may be seen at the periphery of the lesions near the shoulder.

Q. Write short note on complications of atherosclerosis.

Complicated plaques: Atherosclerotic plaques can undergo the following clinically important changes:

Complications of atherosclerosis:

1. Rupture
2. Hemorrhage into the plaque
3. Thrombosis
4. Embolism
5. Aneurysm
6. Calcification.

1. **Rupture, ulceration, or erosion:** Plaque protrudes into the lumen and can disturb the blood flow → resulting in turbulent flow of blood → which can damage the endothelium → cause rupture, ulceration or erosion of the intimal surface of plaques.
2. **Hemorrhage into the plaque:** It may occur due to rupture of the fibrous cap of the plaque or of the thin-walled vessels formed due to neovascularization.
3. **Thrombosis and embolism:** Ulceration/erosion/rupture of endothelial surface → exposes the blood to highly thrombogenic subendothelial collagen → favors

thrombus formation → can partially or completely occlude the lumen (depending on the size of the lumen) → lead to ischemia. The thrombus may become organized or fragment to form thromboemboli.

4. **Atheroembolism:** Plaque rupture → discharge atherosclerotic debris into the bloodstream → results in atheroemboli.
5. **Aneurysm formation:** Atherosclerosis even though an intimal disease may cause pressure or ischemic atrophy of the underlying media. It may also damage the elastic tissue and cause weakening the wall → result in aneurysmal dilation → which may rupture.
6. **Calcification:** It may occur in the central necrotic area of the plaque (dystrophic calcification).

Major clinical consequences of atherosclerosis (Fig. 14.4):

1. Myocardial infarction (heart attack)
2. Cerebral infarction (stroke)
3. Aortic aneurysms
4. Peripheral vascular disease (gangrene of the legs).

Stable plaque:

1. Have thick densely collagenized fibrous cap
2. Minimal inflammation
3. Negligible lipid core.

Vulnerable plaque:

1. Contain numerous foam cells
2. Abundant extracellular lipid
3. Many inflammatory cells
4. Thin fibrous cap
5. Few smooth muscle cells
6. High-risk to undergo rupture.

Clinicopathologic Manifestations of Atherosclerosis

1. Atherosclerotic stenosis

- Atherosclerotic plaques reduce the size of the lumen of the involved vessel. When reduction of the lumen is

sufficiently severe it may lead to tissue ischemia and this lesion is termed as **critical stenosis**.

- The effects of vascular stenosis depend on arterial supply and the metabolic demand of the affected tissue. In small arteries, it may reduce blood flow leading to ischemic injury. In the coronary artery atherosclerotic lesion when produces a 70% decrease in luminal cross-sectional area, the patient may develop chest pain with exertion (*stable angina*; refer Chapter 15). In other sites, diminished arterial blood flow can cause ischemia of the bowel (mesenteric occlusion), sudden cardiac death, chronic ischemic heart disease, ischemic encephalopathy, and intermittent claudication (diminished perfusion of the extremities).

2. Acute plaque change (refer Chapter 15 page 394)

Atheromatous plaques causing myocardial infarction and other acute coronary syndromes are often asymptomatic. Acute plaque change is the sudden change/event **occurring in an atheromatous plaque**. The composition of plaques is dynamic and it decides the risk of rupture. **Vulnerable plaques** are more likely to undergo rupture. These are plaques that have (1) necrotic centre with large areas of foam cells and extracellular lipid, (2) thin fibrous caps or contain few smooth muscle cells or (3) have clusters of inflammatory cells.

- **Mechanical strength and stability of plaque:** Depends mainly on the collagen in the fibrous cap. The balance between synthesis of collagen against its degradation decides the integrity of fibrous cap integrity.
 - ♦ **Collagen synthesis:** It is mainly by smooth muscle cells and loss of these cells reduces the integrity and strength of fibrous cap.
 - ♦ **Collagen degradation:** Generally inflammatory cells in the plaque increase in collagen degradation and reduced collagen synthesis. This reduces the mechanical strength and integrity of the fibrous cap. Statins reduce circulating cholesterol levels and also stabilize plaques by reducing inflammation in plaque.

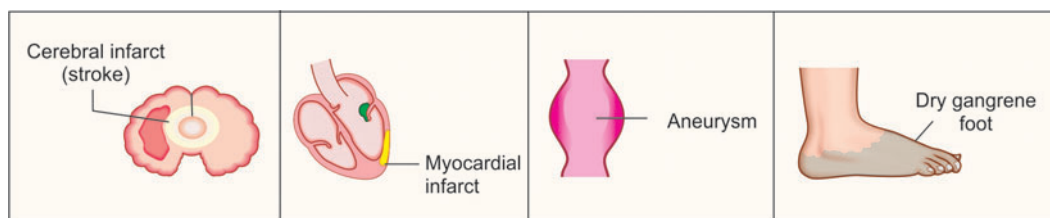


Fig. 14.4: Major clinical consequences of atherosclerosis

Major vessels involved in atherosclerosis

- Large elastic arteries (e.g. the aorta, carotid, and iliac arteries)
- Large and medium-sized muscular arteries (e.g. coronary and popliteal arteries).

- **Extrinsic factors:** Also contribute plaque changes.
 - ◆ Adrenergic stimulation raise systemic blood pressure or induce local vasoconstriction and increase the physical stresses on a given plaque.
 - ◆ Emotional stress can also contribute to plaque disruption.
- 3. **Thrombosis:** Partial or total thrombosis over a disrupted plaque is observed in acute coronary syndromes. Mural thrombi in a coronary artery can undergo embolization.
- 4. **Vasoconstriction:** Vasoconstriction reduces the size of arterial lumen and increases the local mechanical forces. This can cause disruption of plaque.

ANEURYSMS AND DISSECTION

Q. Define aneurysm.

Definition: An aneurysm is defined as a congenital or acquired, **localized, abnormal, permanent dilation of a blood vessel or the heart**.

Classification

Q. Write short note on aneurysm.

Aneurysms are classified by origin, location, gross appearance, composition of vessel wall, and etiology.

Aneurysm: Localized, abnormal, permanent dilation of a blood vessel or the heart.

Aneurysms: Congenital or acquired.

1. **Origin:** It can be congenital or acquired.
2. **Location:** It refers to the type of vessel involved:
 - **Artery** and the specific region involved, such as the aorta or popliteal artery
 - **Vein**
 - **Heart.**

3. Depending on gross appearance (shape and size) (Fig. 14.5):

- **Fusiform aneurysm:** It is ovoid or fusiform dilation of vessel wall that is parallel to the long axis. Size varies in diameter (up to 20 cm) and in length. It can involve aortic arch, abdominal aorta, or iliac arteries.
- **Saccular aneurysm:** It is a localized spherical out-pouchings from the portion of the vessel wall. The size varies from 5 to 20 cm in diameter and the lumen may contain thrombus.
- **Cylindrical aneurysm:** It has parallel dilatation.
- **Arterial dissection/dissecting hematoma:** It develops when blood enters/dissects between the layers of the arterial wall. Just like a hematoma it separates the layers of the arterial wall.
- **Arteriovenous (racemose) aneurysm:** It is a direct communication between an artery and a vein.

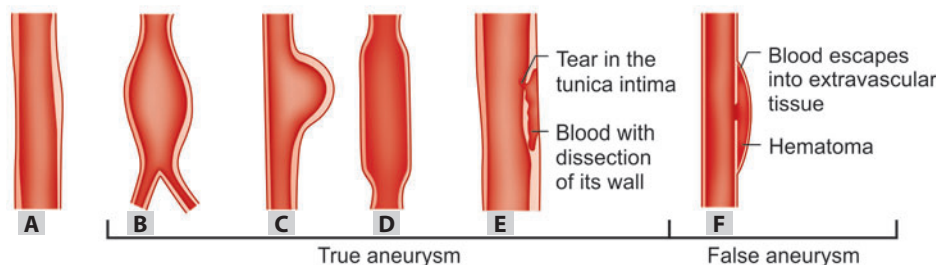
Morphological types of aneurysm:

1. Fusiform
2. Saccular
3. Cylindrical
4. Aortic dissection.

True aneurysm: Involves all 3 layers of the artery or thinned wall of the heart.

4. Depending on the composition of the wall of the aneurysm:

- **True aneurysm** is composed of all the layers of thinned arterial wall (intima, media and adventitia) or attenuated ventricular wall of the heart.
 - Examples: Atherosclerotic, syphilitic, and congenital vascular aneurysms, and ventricular aneurysms that complicates transmural myocardial infarctions.
- **False aneurysm** (or pseudoaneurysm) is a defect in the vascular wall with a hematoma (blood-filled space forms around a blood vessel) which



Figs 14.5A to F: Gross types of aneurysms. (A) Normal blood vessel; (B) Fusiform aneurysm; (C) Saccular aneurysm; (D) Cylindrical aneurysm; (E) Dissecting hematoma; (F) False aneurysm

freely communicates with the intravascular space (“**pulsating hematoma**”). It usually develops after traumatic rupture or a perforating injury.

- Examples: Rupture of left ventricle, which complicates myocardial infarction or hematoma that follows trauma to artery.
- **Arterial dissection** is characterized by entry of blood into the arterial wall through an intimal defect, which separates the underlying layers.

Q. List the causes of aneurysm.

5. Depending on the etiology:

- Atherosclerotic aneurysm
- Syphilitic aneurysm
- Dissecting hematoma
- Mycotic aneurysm
- Berry aneurysm.

Pathogenesis of Aneurysms

Aneurysm develops due to weakening of the vessel wall either due to:

1. Inadequate abnormal synthesis of connective tissue of the vessel wall or
2. Increased degradation of connective tissue.

Aneurysms develop when there is weakening of vessel walls, which may be inherited or acquired. The loss of balance between connective tissue synthesis and degradation of the vascular wall may produce aneurysm.

1. **Inadequate or abnormal synthesis of connective tissue of the vascular wall:** TGF- β regulates smooth muscle cell proliferation and synthesis of connective tissue matrix. Mutations in TGF- β receptors or downstream signaling pathways \rightarrow defective synthesis of elastin and collagen \rightarrow aneurysm.
 - **Marfan syndrome** is characterized by defective synthesis of the **fibrillin** \rightarrow weakening of the aortic wall \rightarrow aneurysm.
 - **Ehlers-Danlos syndrome** is associated with defective type III collagen synthesis \rightarrow aneurysm.
 - **Vitamin C (ascorbate) deficiency** \rightarrow altered collagen cross-linking.

Ehlers-Danlos syndrome: Defective synthesis of type III collagen.

Marfan syndrome: Defective synthesis of the fibrillin.

2. **Increased degradation of connective tissue:** Increased production of matrix metalloproteinases (MMP) enzymes (e.g. by macrophages in atherosclerosis)

can degrade the ECM in the arterial wall may cause aneurysm formation. This may be seen in atherosclerotic plaque or in vasculitis.

- **Loss of smooth muscle cells:** It may occur **due to ischemia** of the inner media as in atherosclerosis or ischemia of the outer media as in **systemic hypertension**. This results in degenerative changes in the aorta \rightarrow fibrosis, increased synthesis of ECM, and accumulation of excessive amounts of amorphous proteoglycans in the medial wall. These changes in the aortic wall are called as **cystic medial degeneration**. These changes are nonspecific and can be seen in numerous conditions including Marfan disease and scurvy.

Predisposition Factors

1. **Atherosclerosis:** It predisposes to aneurysms in the abdominal aorta.
2. **Hypertension:** It predisposes to aneurysms of the ascending aorta.
3. **Others:**
 - **Trauma**
 - **Vasculitis**
 - **Congenital defects** (e.g. berry aneurysms in the circle of Willis)
 - **Tertiary syphilis:** It is a rare cause of aortic aneurysms. Treponemas has affinity for vasa vasorum of mainly thoracic aorta \rightarrow obliterative endarteritis \rightarrow leads to ischemic damage to the aortic media and aneurysm (syphilitic mesoaortitis)
 - **Infections (mycotic aneurysms):** The term **mycotic** is a **misnomer** and does not indicate that the infection is due to fungus and is usually **bacterial**. The source of infection may be:
 - Septic embolus, usually as a complication of infective endocarditis
 - Extension of an adjacent suppurative process
 - Circulating organisms directly infecting the arterial wall.

Fungus that invade blood vessel: *Aspergillus*, *Candida* and *Mucor*.

Bacteria that invade blood vessel: *B. fragilis*, *P. aeruginosa* and *Salmonella*.

Abdominal Aortic Aneurysm

Gender and age: Abdominal aortic aneurysm (AAA) is more common **in men, in smokers**, and rare before the age of 50 years.

Causes

Atherosclerosis is the major cause of abdominal aortic aneurysm.

1. **Atherosclerotic AAA:** It is a **major cause**.
 - Atherosclerotic plaque in the intima causes thinning of media and reduces the diffusion of nutrient and waste from the lumen of vessel into the arterial wall. The media undergoes necrosis and cause **weakness and thinning of arterial wall**.
 - The **excessive degradation of ECM by MMP** secreted from inflammatory cell infiltrates mainly macrophages present in the plaque.
2. **Inflammatory AAA:** It is characterized by dense periaortic fibrosis accompanied by inflammatory cells (lymphocytes, plasma cells and macrophages).
3. **Mycotic AAA:** It is due to **infection of the aortic wall by circulating microorganisms** (e.g. bacteremia from a primary *Salmonella* gastroenteritis). The suppurative process destroys the media → aneurysm → rupture.

Morphology of Atherosclerotic Aneurysm

- **Site:** Aneurysm is **usually observed below the origin of renal arteries and above the bifurcation of the aorta**.
- **Appearance:**
 - **Shape:** **Saccular or fusiform**.
 - **Size:** May be up to 15 cm in diameter, and up to 25 cm in length.
 - **Wall of the aorta:** Intimal surface shows severe **complicated atherosclerosis with destruction and thinning of the underlying aortic media**.
 - **Lumen:** It frequently shows a bland, laminated, poorly organized **mural thrombus**.

Clinical Features and Complications

Complications of aneurysm:

1. Rupture
 2. Thrombosis
 3. Embolization.
- **Rupture:** Aneurysm may rupture into the **peritoneal cavity or retroperitoneal tissues** → leading to massive, fatal hemorrhage. The risk of rupture increases as the size increases.
 - **Obstruction of a branch:** It can lead to **ischemia of tissues supplied by the obstructed vessel**. For example Example: iliac (leg), renal (kidney).
 - **Embolism:** Atherosclerotic plaque or mural thrombus may fragment to form emboli.

TABLE 14.2: Clinical presentation in thoracic aortic aneurysm

Encroached structures	Symptoms
Lungs and airways	Respiratory difficulties
Esophagus	Difficulty in swallowing
Irritation of or pressure on the recurrent laryngeal nerves	Persistent cough
Erosion of bone (i.e. ribs and vertebral bodies)	Pain

- **Compression of an adjacent structure:** For example, compression of a ureter.
- **Abdominal mass:** It may simulate a tumor.

Thoracic Aortic Aneurysms

Causes: Hypertension, Marfan syndrome, other disorders associated with mutations in TGF- β signaling pathways, and syphilis (not observed nowadays).

Clinical presentation: Symptoms depend on the encroached structure (Table 14.2).

Other symptoms:

- Aortic valve dilation and valvular insufficiency.
- Narrowing of the coronary ostia → cause myocardial ischemia.
- Rupture of aneurysm → fatal.
- Syphilitic aneurysms → heart failure due to aortic valvular incompetence.

Syphilitic Aneurysm

Q. Write short note on syphilitic aortitis.

Syphilitic aneurysms: Meso-aortitis → aortic aneurysm and aortic regurgitation.

- Syphilis causes aortitis and aneurysms
- Mainly affect the ascending aorta
- **Microscopy:** It shows **endarteritis and periarteritis** (lymphocytes, plasma cells, and macrophages) of vasa vasorum. Obliterative endarteritis → cause focal necrosis and scarring of the media
- Medial scars → produce roughened intimal surface → **tree bark appearance**
- Weakened wall of the ascending aorta and aortic arch → **fusiform aneurysm**.

Aortic Dissection

Q. Write short note on dissecting aneurysm/hematoma/aortic dissection.

Aortic dissection: Associated with cystic medial degeneration.

Definition: Aortic dissection develops when **blood from the aortic lumen enters into the aortic wall** and travels along the layers of the media to form a blood-filled channel within the aortic wall.

Aortic dissection may or may not be associated with aortic dilation. **Older term “dissecting aneurysm” is replaced by aortic dissection.**

It is fatal, if the dissection ruptures through the adventitia → results in hemorrhages into adjacent spaces.

Etiology

Aortic dissection: Blood enters the wall of aorta and separates its layers.

Occurs mainly in two groups: (1) men between **40 and 60 years, with hypertension** or (2) **younger patients with connective tissue disease** of the aorta, e.g. **Marfan syndrome**.

- **Iatrogenic:** It may develop as a complication of **arterial cannulations** during diagnostic catheterization or cardiopulmonary bypass.
- **During or after pregnancy:** Cause not known.

Pathogenesis

- **Hypertension:** It is the **major risk factor**. Aortas show medial hypertrophy of the vasa vasorum, degenerative changes in the aortic media and loss of medial smooth muscle cells.
- **Inherited or acquired connective tissue disorders:** It may be responsible in few cases. For example: **Marfan syndrome, Ehlers-Danlos syndrome, vitamin C deficiency, copper metabolic defects**.
- An **aortic dissection usually starts with a tear in the intima**. The trigger for the intimal tear is not known.

MORPHOLOGY

- **Site of intimal tear:** It is in the **ascending aorta**, usually within 10 cm of the aortic valve (majority of patients).
- **Characteristic of tear:** (1) **Transverse or oblique**, (2) 1–5 cm in length, and (3) sharp with jagged edges.
- **Site of dissection:** It is usually **between the middle and outer thirds of the tunica media**.
- **Extent of involvement:** It can extend proximally along the aorta toward the heart and distally into the iliac and femoral arteries.

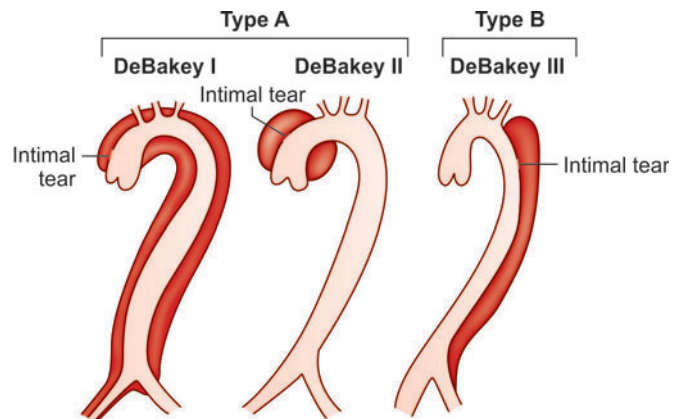


Fig. 14.6: Classification of aortic dissections. Type A (proximal) involves the ascending aorta (DeBakey I and II), Type B (distal or DeBakey III) dissections arise beyond the subclavian artery

Classification (Fig. 14.6)

Two types:

1. **Type A dissections:** They have **proximal lesions** involving either **both the ascending and descending aorta or only the ascending aorta** (types I and II of the DeBakey classification). It is more common but dangerous.
2. **Type B dissections:** They have **distal lesions** which **do not involve the ascending part** and usually begin **distal to the subclavian artery** (DeBakey type III).

Dr Michael DeBakey is a pioneer in vascular surgery.

Aortic dissection:

- Type A dissections (DeBakey type I and II)
- Type B dissections (DeBakey type III).

Complications due to Rupture

- **Rupture:** Rupture occurs through the tunica adventitia, may result in:
 - **Massive hemorrhage** (e.g. into the thoracic or abdominal cavities) or
 - **Cardiac tamponade** (hemorrhage into the pericardial sac).
 - **Double-barreled aorta:** It may develop in few cases, when the **dissecting hematoma reenters the lumen of the aorta through a second distal intimal tear**. This creates a new false vascular channel and prevents the fatal extra-aortic hemorrhage.
- **Microscopy:** In most cases, do not show any identifiable changes in the aortic wall. The most frequent microscopic feature is **cystic medial degeneration** without any inflammation.

Classical Symptoms

Aortic dissection: Complications are due to rupture or obstruction of branches of aorta.

1. **Sudden onset of severe pain**, beginning in the anterior chest, radiating to the back between the scapulae, and moving downward as the dissection progresses.
2. Pain may be **misdiagnosed as that of myocardial infarction**.

Cause of Death

1. **Rupture of the dissection** outward into the pericardial, pleural, or peritoneal cavities.
2. **Retrograde dissection** into the aortic root can cause aortic valvular insufficiency, cardiac tamponade, and myocardial infarction.
3. **Dissection** may also extend **into the great arteries of the neck** or into the coronary, renal, mesenteric, or iliac arteries, causing critical vascular obstruction and ischemic damage.

HYPERTENSIVE VASCULAR DISEASE

Hypertension is a common disorder and maintenance of blood pressures is important to prevent untoward consequences. Low blood pressure (hypotension) can cause inadequate perfusion of organ perfusion leading to tissue dysfunction or death. High blood pressure (hypertension) can cause end-organ damage and is one of the major risk factors for atherosclerosis.

WHO definition of hypertension: Hypertension is defined as **systolic pressure above 160 mm Hg and/or diastolic pressure above 90**.

Causes of Hypertension (Box 14.1)

- **Primary/essential/idiopathic** hypertension: It constitutes about 95% of cases.
- **Secondary** hypertension: It forms about 5% of cases and there is an identifiable cause.

The prevalence and vulnerability to complications of hypertension increase with age.

Regulation of Normal Blood Pressure

Normal blood pressure is essential for survival; it causes damage when it increases continuously above the normal range.

BOX 14.1: Major causes of hypertension (systolic and diastolic)

Hypertension: Common disorder which may be idiopathic or secondary to other disorders.

PRIMARY/ESSENTIAL HYPERTENSION (~ 95% of case)

Secondary hypertension

Renal

- Acute glomerulonephritis
- Chronic renal disease
- Polycystic kidney disease
- Renal artery stenosis (renovascular hypertension)
- Renin producing tumors
- Obstructive uropathy

Endocrine

- Adrenal disorders: Cushing's syndrome, primary aldosteronism
- Pheochromocytoma
- Exogenous hormones: Glucocorticoids, estrogen
- Pregnancy-induced: Pre-eclampsia/eclampsia
- Others: Hypothyroidism (myxedema), hyperthyroidism (thyrotoxicosis), hypercalcemia, acromegaly

Cardiovascular

- Coarctation of aorta
- Polyarteritis nodosa
- Increased cardiac output
- Increased intravascular volume

Neurologic

- Psychogenic
- Increased intracranial pressure
- Sleep apnea
- Acute stress: e.g. surgery

Factors Determining Blood Pressure

Blood pressure: Depends on cardiac output and vascular resistance.

Vascular resistance in the arterioles: Depends on neural and hormonal mechanisms.

- The major factors include age, sex, body mass index and diet, particularly sodium intake, exertion, emotional state and others.
- Blood pressure depends on two hemodynamic variables, namely **cardiac output** and **peripheral vascular resistance** (Fig. 14.7). These two, in turn, are influenced by many genetic, environmental and demographic factors.
 - **Cardiac output:** It depends on stroke volume, and heart rate. Stroke volume in turn is influenced by the sodium homeostasis. Heart rate and contractility of myocardium (affects stroke volume) are regulated

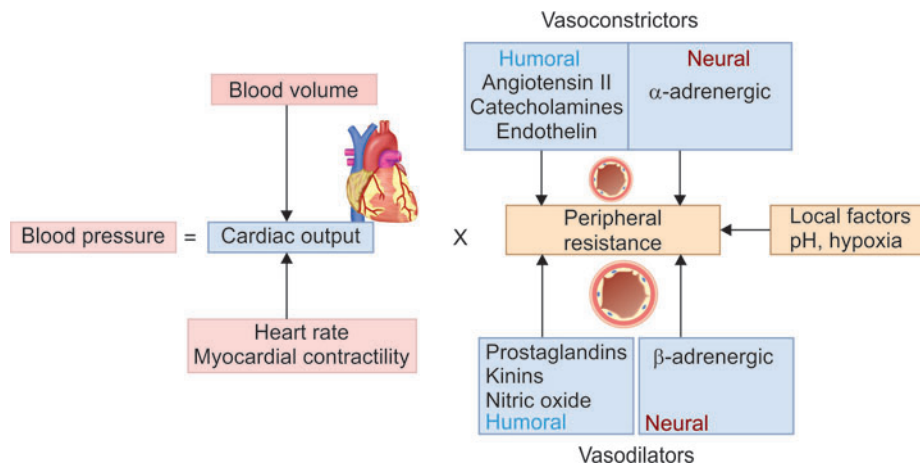


Fig. 14.7: Regulation of blood pressure

by the α- and β-adrenergic systems (also effects on vascular tone).

- **Peripheral vascular resistance:** It is determined by functional and anatomic changes in small arteries and arterioles. Vascular tone depends on the balance between vasoconstrictors and vasodilators. Blood pressure is also influenced by tissue pH and hypoxia.

Cardiac output depends on:

- Heart rate
- Stroke volume.

Stroke volume depends on blood volume.

Role of Kidney

The kidneys **play an important role in the regulation of blood pressure.**

Blood volume regulated by: Sodium excretion or re-absorption by the kidney.

Kidney: Plays an important role in the regulation of blood pressure.

- **Renin-angiotensin system:** Kidney influences both peripheral vascular resistance and sodium homeostasis (thereby blood volume) through the renin-angiotensin system.
 - **Renin:** Whenever there is a fall in blood pressure, renin is secreted by the juxtaglomerular cells of the kidney and released into the blood circulation. It cleaves plasma angiotensinogen to angiotensin I, which is then converted to angiotensin II by angiotensin converting enzyme.
 - **Angiotensin II:** It raises blood pressure by increasing both peripheral resistance (direct action on vascular

smooth muscle cells and causes vasoconstriction) and **blood volume** (by stimulating secretion of aldosterone by the adrenal zona glomerulosa, and increased reabsorption of sodium in distal tubules).

- **Antihypertensive substances:** The kidney also produces substances which causes vasodilatation and have antihypertensive effect. These include prostaglandins and nitric oxide (NO). They counterbalance the vasoconstriction produced by angiotensin II.
- **Sodium homeostasis and blood volume:**
 - **When blood volume is reduced,** the glomerular filtration rate falls → leads to increased reabsorption of sodium by proximal tubules of kidney → thereby conserves sodium and expands blood volume.
 - **When blood volume is increased,** natriuretic factors (natriuretic peptides) are secreted by atrial and ventricular myocardium. They inhibit sodium reabsorption in distal tubules and cause excretion of sodium and diuresis. Natriuretic peptides also cause vasodilation and may be considered as endogenous inhibitors of the renin-angiotensin system.

Pathogenesis of Hypertension

Mechanisms of Essential Hypertension

Q. Write short note on pathogenesis of hypertension.

Essential hypertension is a **complex and multifactorial disorder**. Specific trigger is not known. Probable factors that play a significant role includes:

1. **Decreased renal sodium excretion:** It is probably the **key feature**. Decreased excretion of sodium by kidney → leads to an **increase in fluid volume, cardiac output, and peripheral vasoconstriction** → raises the blood pressure.

2. **Raised vascular resistance:** Factors that produce vasoconstriction or stimuli that cause structural changes in the vessel wall → result in an increase in peripheral vascular resistance → cause primary hypertension.
3. **Genetic factors:** They play an important role in the development of hypertension. The genetic defects may be in the enzymes involved in aldosterone metabolism, sodium reabsorption and smooth muscle cell growth.
4. **Environmental factors:** These include stress, obesity, smoking, lack of physical activity and heavy intake of sodium salt.

Essential hypertension:

1. Reduced renal excretion of sodium
2. Increased vascular resistance
3. Genetic factors
4. Environmental factors.

Essential hypertension: Accounts for ~95% of cases and is a multifactorial disorder.

Angiotensin II:

1. Increases vascular smooth muscle tone
2. Increases aldosterone secretion by adrenal → increases sodium absorption by kidney.

Consequences of Hypertension

Hypertension: One of the modifiable risk factor for atherosclerosis. It may predispose to cardiac hypertrophy, congestive heart failure and renal failure.

- **Risk factor:** Hypertension is one of the major modifiable risk factors for **atherosclerosis**.
- **Lesions/diseases produced:** Cardiac hypertrophy, congestive heart failure (hypertensive heart disease) and ischemic heart disease (IHD), multi-infarct dementia, aortic dissection, stroke, subarachnoid hemorrhage, hypertensive encephalopathy, retinal ischemia/infarction and renal failure.
- **Accelerated or malignant hypertension:** It is characterized by **rapid raise in blood pressure** (i.e. systolic pressure over 200 mm Hg, diastolic pressure over 120 mm Hg) → **renal failure, and retinal hemorrhages** and exudates, **with or without papilledema**.
 - It may develop in normotensive individuals but more common in patients with preexisting benign hypertension (essential or secondary).
 - It develops in ~5%, of hypertensive persons. If not treated, death occurs within a year or two.

Pathogenesis of Secondary Hypertension

Renin: Major regulator of blood pressure is secreted by the kidneys when the blood pressure is decreased in the afferent arterioles.

• Mechanism of renovascular hypertension:

- Renal artery stenosis → decreased glomerular flow and pressure in the afferent arteriole of the glomerulus → stimulates renin secretion and production of angiotensin II → vasoconstriction → increased peripheral resistance.
- Renal artery stenosis also increases sodium reabsorption → increases blood volume through the aldosterone mechanism.

- **Primary hyperaldosteronism:** It is one of the most common causes of secondary hypertension.

Morphology of Vascular Changes in Hypertension

Q. Write short note on vascular pathology in hypertension.

Large and Medium Vessel Disease:

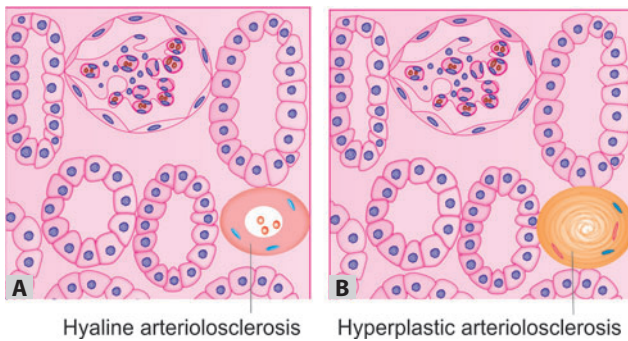
Atherosclerosis

- Hypertension is one of the **major modifiable risk factor** for **atherogenesis**.
- Causes **degenerative changes in the walls** of large and medium arteries.
- Predisposes to: (1) **Aortic dissection** and (2) **Cerebrovascular hemorrhage**.

Small Vessel Diseases

Two forms can occur in hypertension: (1) Hyaline arteriosclerosis, and (2) hyperplastic arteriosclerosis.

1. **Hyaline arteriosclerosis:** It is seen in the arterioles in patients with benign hypertension.
 - **Microscopy:** It shows **thickening of the wall due to homogeneous, pink hyaline material** and **narrowing of the lumen** (Fig. 14.8A).
 - **Mechanism:** Chronic hemodynamic stress produced by hypertension → produces **leakage of protein into the vessel wall** → **increased synthesis ECM by smooth muscle cell** → **hyalinization** of the wall of arteriole.
 - **Consequence:** The arteriolar narrowing → impaired blood supply to kidney → produces **ischemia and glomerular scarring**. The kidney changes in benign hypertension are called **benign nephrosclerosis**.



Figs 14.8A and B: Vascular changes (in renal blood vessels) in hypertension. (A) Hyaline arteriosclerosis in benign hypertension: The arteriolar wall is thickened, hyalinized, and the lumen is narrowed; (B) Hyperplastic arteriosclerosis in malignant hypertension: shows onion-skinning with obliteration of arteriolar lumen

Causes of hyaline arteriosclerosis:

1. Benign hypertension
 2. Diabetic microangiopathy
 3. Old age (either normo- or hypertensive).
2. **Hyperplastic arteriosclerosis:** It occurs in **severe (malignant) hypertension**.
- **Microscopy:** The blood vessels show “onion-skin,” **concentric, laminated thickening of the arteriolar walls** and narrowing of the arteriolar lumen (Fig. 14.8B). It may also show **fibrinoid deposits and necrosis of vessel wall (necrotizing arteriolitis)**, particularly in the kidney.

Blood vessels in malignant hypertension:

- Hyperplastic arteriosclerosis (onion-skin appearance)
- Fibrinoid necrosis—necrotizing arteriolitis.

VASCULITIS

Vasculitis: Inflammation and damage of the blood vessel.

Definition: Vasculitis is a **heterogeneous group** of disorders **characterized by inflammation and damage of the blood vessel**. The lumen of the involved vessel is usually narrowed → may lead to ischemia of the tissues supplied.

General Characteristics

- **Primary or secondary:** Vasculitis may be primary or secondary component of another primary disease.
- **Vessel involved:** It can involve **any type, size, and location** of blood vessel. Mostly, involve small vessels (arterioles to capillaries to venules).
- **Organ involved:** Vasculitis may be **restricted to one organ** (e.g. skin), or **may involve many organ systems**.

Classification

May be classified in many ways such as: (1) According to size of the vessel involved (Table 14.3), (2) role of immune complexes, and presence of specific autoantibodies, (3) granuloma formation, and (4) organ specificity.

Pathogenesis of Vasculitis

Two common pathogenic mechanisms are:

1. Immune-mediated mechanism (noninfectious vasculitis).
2. Direct invasion of vascular walls by infectious pathogens (infectious vasculitis).

Infections can also indirectly induce noninfectious vasculitis by generating immune complexes or triggering cross-reactivity.

Noninfectious Vasculitis

Mechanism: Main immunological mechanisms of noninfectious vasculitis are: (1) Immune complex mediated, (2) antineutrophil cytoplasmic antibody-mediated, and (3) antiendothelial cell antibody-mediated.

Immune Complex-mediated Vascular Injury

Immune complex-mediated vascular injury is due to type III hypersensitivity reaction.

Immune complex-mediated tissue vasculitis is characterized by deposition immune complexes in the vessel walls. The antigen in the immune complex is not known in most of the cases.

Mechanism of tissue damage:

- The **antigen-antibody (immune) complexes are formed** when there is little antigen excess.
- The immune complexes get **deposited in vessel walls** → activate complement system (e.g. C5a) → chemotactic for neutrophils → phagocytose the immune complexes → release their contents → **damage the vessel wall**.
- As the process becomes chronic, mononuclear cells infiltrate the vessel wall.
- The lumen of the involved vessel is narrowed → lead to ischemic changes in the tissues supplied by the involved vessel (refer page 124-127).

Causes of immune-mediated vasculitis:

- **Systemic immunological diseases:** For example, **systemic lupus erythematosus (SLE)** and **polyarteritis nodosa**.
- **Drug hypersensitivity:** Drugs themselves may be foreign proteins (e.g. streptokinase) or they bind to serum proteins (e.g. penicillin) and behave like antigens.

TABLE 14.3: Classification of vasculitides according to blood vessels involved

Examples	Organs/vessels involved	Microscopic features	
Predominantly involving large vessels (aorta and large branches to extremities, head, and neck)			Vasculitis with granulomatous lesions: <ul style="list-style-type: none"> • Wegener's granulomatosis • Buerger's disease • Takayasu's arteritis • Giantcell arteritis • Churg-Strauss syndrome.
Giantcell (temporal) arteritis	Temporal artery and aorta. Patients older than age 50	Granulomatous inflammation with giant cells	
Takayau's arteritis	Aorta, aortic arch and major branches, and pulmonary arteries Patients younger than age 50	Granulomatous with some giant cells → fibrosis in chronic stages	
Predominantly involving medium vessels (visceral arteries and their branches)			Small vessel vasculitis: <ul style="list-style-type: none"> • Wegener's granulomaosis • Churg-Strauss syndrome • Microscopic polyangiitis.
Polyarteritis nodosa (PAN)	Skin, peripheral nerve, GI tract, and renal, etc. Spares pulmonary vessels	Fibrinoid necrosis, with mixed cellular infiltrate	
Kawasaki disease	Arteritis with mucocutaneous lymph node syndrome. Usually occurs in children	Similar to PAN, fibrinoid necrosis less prominent	
Predominantly involving small vessels (arterioles, venules, and capillaries)			ANCA: Autoantibodies against certain proteins in the cytoplasmic granules of neutrophil and monocytes.
Wegener's granulomatosis	Upper respiratory tract, lungs, kidneys, skin, and eyes	Necrotizing or granulomatous (or both); mixed cellular infiltrate	
Churg-Strauss syndrome	Upper respiratory tract, lungs, heart, peripheral nerves	Necrotizing or granulomatous (or both); prominent eosinophils	
Microscopic polyangiitis	Necrotizing glomerulonephritis and pulmonary capillaries	Leukocytoclastic, with occasional eosinophils	

- **Secondary to viral infections:** Viral proteins may form immune complexes. Examples:
 - **Hepatitis B virus (HBV) associated polyarteritis nodosa (PAN).**
 - Cryoglobulinemic vasculitis is strongly associated with hepatitis C virus (HCV) infection.

Hypersensitivity vasculitis most commonly involves: Post-capillary venules.

Antineutrophil Cytoplasmic Antibodies

Antineutrophil cytoplasmic antibodies (ANCA) are heterogeneous group of **autoantibodies directed against certain proteins (mainly enzymes) in the cytoplasmic granules of neutrophils and monocytes**. Two important ANCAs are:

1. **Anti-myeloperoxidase (MPO-ANCA):** MPO is a lysosomal enzyme normally involved in producing oxygen-free radicals. MPO-ANCAs can be induced by drugs (e.g. propylthiouracil). They are associated with **microscopic polyangiitis and Churg-Strauss syndrome**.
2. **Anti-proteinase-3 (PR3-ANCA):** PR3 is a constituent of neutrophil azurophilic granule. It shares homology with many microbial peptides and antibodies against microbial peptides may form PR3-ANCAs. They are associated with **Wegener's granulomatosis**.

MPO-ANCA were previously called p-ANCA.

PR3-ANCA were previously called c-ANCA.

Antiendothelial Cell Antibodies

They can produce **endothelial cell injury and lysis through either complement-mediated cytotoxicity or antibody-dependent cellular cytotoxicity**. For examples, Kawasaki disease and systemic lupus erythematosus (SLE).

Few examples of vasculitis are considered here.

Antiendothelial antibodies:

- Kawasaki disease
- SLE.

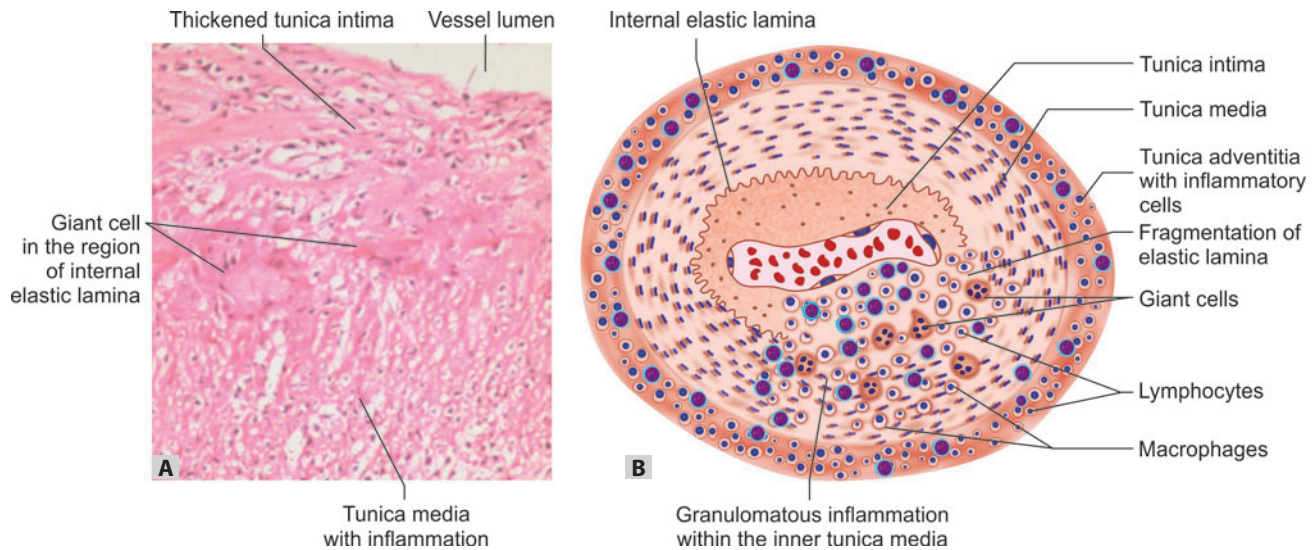
Giant Cell (Temporal) Arteritis

Temporal arteritis is also known as giant cell arteritis.

Definition: Giant cell (temporal/cranial) arteritis is a **chronic, typically granulomatous inflammation of medium- and large-sized arteries**.

Vessels involved: One or more branches of the carotid artery (e.g. **temporal artery**). Can involve multiple arteries, aorta (giant cell aortitis) and its branches.

Pathogenesis: T-cell mediated immune response against an unknown antigen. Proinflammatory cytokines (TNF) and antiendothelial antibodies may also be involved.



Figs 14.9A and B: Microscopic features. (A) H and E staining; (B) Diagrammatic representation of giant cell arteritis

MORPHOLOGY

- **Gross:** Involved segment of the artery show **nodular thickening of the intima** → **reduces the size of lumen** → **ischemia**.
- **Microscopy** (Fig. 14.9):
 - **Granulomatous inflammation** within the **inner tunica media** centered on the internal elastic lamina. It shows multinucleated giant cells (either Langhans type or foreign body type).
 - **Fragmentation of elastic lamina**.
 - **Chronic inflammation** consisting of T-cells and macrophages.
 - **Segmental distribution of inflammation** with segments of relatively normal artery between the lesions.
 - **Intimal fibrosis** in healed stage.

Clinical Features

- Manifest **after the age of 50**.
- **Fever, anemia, fatigue, headache** (most severe along the course of the superficial temporal artery), and weight loss.
- On palpation, **the involved artery is tender, thickened, or nodular**.

Diagnosis: It is confirmed by **biopsy of the temporal artery**. Responds well to corticosteroids and anti-TNF therapy.

Giant cell arteritis:

- Medium and large-sized arteries
- Granulomatous inflammation
- Fragmentation of internal elastic lamina
- Immunologically-mediated chronic inflammation.

Giant cell arteritis: Biopsy of the involved artery is the investigation of choice.

Polyarteritis Nodosa

Polyarteritis nodosa: Systemic, necrotizing vasculitis of small and medium-sized muscular arteries.

Definition: Polyarteritis nodosa (PAN) is a **systemic, necrotizing vasculitis of small and medium-sized muscular arteries** characteristically involving the **renal and visceral arteries**.

It **sparcs the smallest blood vessels** (arterioles, venules, and capillaries). PAN **does not involve pulmonary arteries**.

Etiology: Cause not known in the majority of cases. About 30% of patients with PAN have **chronic hepatitis B with HBsAg-HbsAb complexes** in affected vessels. This indicates an **immune complex-mediated** etiology in this subset.

MORPHOLOGY

Polyarteritis nodosa does not involve pulmonary artery and spares small blood vessels.

- **Vessels involved:** Vessels of the skin, kidneys, heart, liver, peripheral nerves, and gastrointestinal tract.
- **Gross:** **Nodular lesions** mainly at the points of arterial branchings. **Aneurysms** may also be seen.
- **Microscopy** (Fig. 14.10): Lesions are **segmental** (involve only part of the vessel circumference and at branch points of arteries).
 - **Transmural necrotizing inflammation of small to medium-sized arteries** → **weakens the wall of artery** → can lead to aneurysms or rupture.
 - **Acute phase:**
 - ♦ **Transmural inflammation** of the arterial wall and perivascular infiltration by **polymorphonuclear neutrophils** and eosinophils.

- **Acute phase:**
 - ♦ **Transmural inflammation** of the arterial wall and perivascular infiltration by **polymorphonuclear neutrophils** and eosinophils.
 - ♦ **Fibrinoid necrosis**
 - ♦ **Thrombosis** → infarction of the tissues supplied by the involved vessel.
 - ♦ **Aneurysm** (up to 1 cm in size) → may rupture → produce hemorrhage.
- **Late phase:**
 - ♦ **Subacute and chronic stages:** Acute inflammatory infiltrate replaced by **mononuclear cells**. **Fibrous thickening of the vessel wall** → further occlusion of the vessel lumen.

PAN:

- Segmental involvement
- Transmural inflammation by neutrophils
- Fibrinoid necrosis
- Thrombus formation.

PAN: Characteristically, the lesions at different stages of development (from early acute to late) coexist in different vessels or even within the same vessel.

Consequences of vasculitis: **Thrombosis** → **impaired perfusion** → lead to ulcerations, infarcts, ischemic atrophy, or hemorrhages in the distribution of affected vessels.

Clinical Features

PAN: Renal arterial involvement is a major cause of death.

- Usually occurs in **young adults**.
- Nonspecific symptoms are malaise, fever, and weight loss.

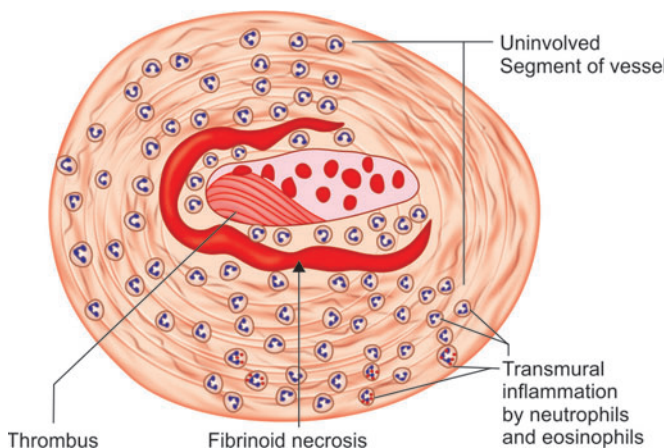


Fig. 14.10: Microscopic features (diagrammatic) of acute phase of polyarteritis nodosa

- Specific complaints are due to **ischemia and infarction of affected tissues and organs**.

- **Renal involvement manifests as hypertension** (usually developing rapidly), renal insufficiency, or hemorrhage due to microaneurysms.
- Gastrointestinal tract involvement present with abdominal pain and melena (bloody stool).

Prognosis: It is fatal, if not treated. Immunosuppression produces remissions or cure in about 90% of patients.

Wegener's Granulomatosis

Definition: Classic Wegener's granulomatosis is a **necrotizing vasculitis**, which involves the **upper respiratory tract**, the **lungs**, and the **kidneys**.

Pathological Hallmarks

1. **Acute necrotizing granulomatous inflammation in the upper respiratory tract** (ear, nose, sinuses, throat) or **the lower respiratory tract** (lung) or both. It may present as chronic sinusitis and mucosal ulcerations of the nasopharynx.
2. **Necrotizing or granulomatous vasculitis** affects **small to medium-sized vessels** (e.g. capillaries, venules, arterioles, and arteries). It may present with persistent pneumonitis and bilateral nodular and cavitary infiltrates.
3. **Renal lesion in the form of focal and segmental necrotizing**, often **crescentic glomerulonephritis**.

ANCA is associated with Wegener's granulomatosis.

Age and gender: It usually presents during **fourth to fifth decade**. **Males** are affected more often than females.

Pathogenesis: Not clear. Probably represents a form of **T-cell-mediated hypersensitivity reaction to an exogenous** (inhaled infectious or other environmental agent) or **endogenous antigen**. About **95% of patients show PR3-ANCA**s, and these autoantibodies may play a role in the pathogenesis of this disease.

MORPHOLOGY

Mainly involves three organs but may be widespread involving any organ such as eyes, skin, kidney, and other organs (e.g. heart).

1. **Upper respiratory tract lesions:** They range from **inflammatory sinusitis** to **ulcerative lesions** in the **nose, palate, or pharynx**.
2. **Lower respiratory tract:** Lung shows **multiple, bilateral, nodular cavitary infiltrates**. Microscopically, it is characterized by:
 - a. **Necrotizing granulomas:** It consists of **geographic patterns of central necrosis** surrounded by a zone of **fibroblastic proliferation with giant cells**, reminiscent of mycobacterial or fungal infections. The granulomas may be **either intravascular or extravascular**.
 - b. **Necrotizing vasculitis of small arteries and veins.**

3. Renal lesions:

- a. **Early lesions:** Glomeruli show **focal and segmental necrotizing glomerulonephritis**.
- b. **Advanced lesions:** They are characterized by **rapidly progressive crescentic glomerulonephritis (RPGN)**.

Main organs involved in Wegener's granulomatosis:

1. Upper respiratory tract
2. Lower respiratory tract
3. Kidney.

Strawberry gums: Wegener's granulomatosis.

Course: If not treated, it is usually rapidly fatal and majority die within 1 year.

VASCULAR TUMORS

Tumors of blood vessels are classified as benign, intermediate grade and malignant (Box 14.2).

Endothelial cells:

- Contain Weibel Palade bodies with von Willebrand factor
- Identified by CD 31, CD34 and vWF.

BOX 14.2: Classification of vascular tumors

Benign neoplasms, developmental and acquired conditions

- Hemangioma: Capillary hemangioma, cavernous hemangioma, pyogenic granuloma

Intermediate-grade neoplasms

- Kaposi sarcoma
- Hemangioendothelioma

Malignant neoplasms

- Angiosarcoma
- Hemangiopericytoma

Hemangioma

Hemangiomas are **very common benign tumors of blood vessels** and form about 7% of all benign tumors of infancy and childhood. Most of these lesions are present from birth and increase in size as the child grows.

Types of hemangioma: Capillary and cavernous.

Capillary Hemangioma

- **Most common type of hemangioma.**
- **Sites**
 - Most commonly in the **skin, subcutaneous tissues**, and mucous membranes of the oral cavities and lips.
 - Internal organs: For example, **liver**, spleen, and kidneys.

MORPHOLOGY

Gross

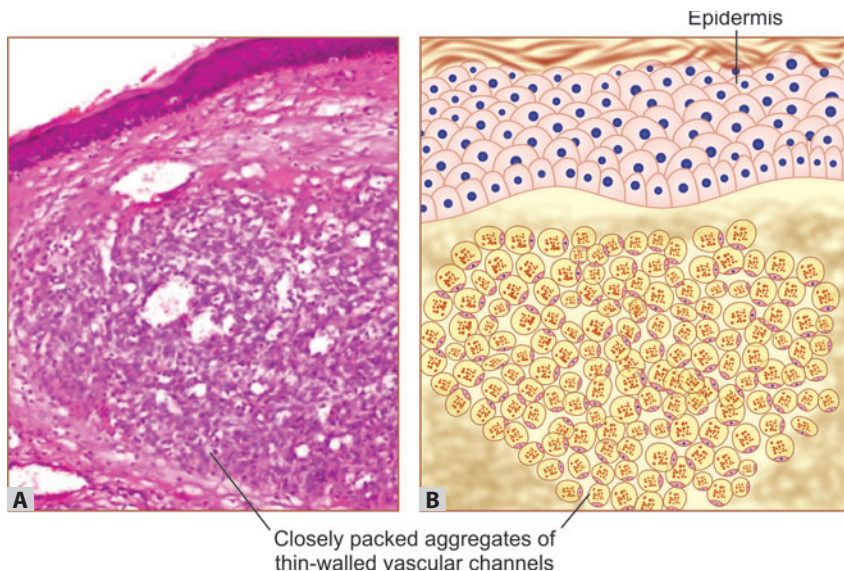
- Bright red to blue in color.
- Size varies from few millimeters to several centimeters in diameter.
- May be in level with the surface of the skin or slightly raised. The skin overlying it is usually intact.

Microscopy (Figs 14.11A and B):

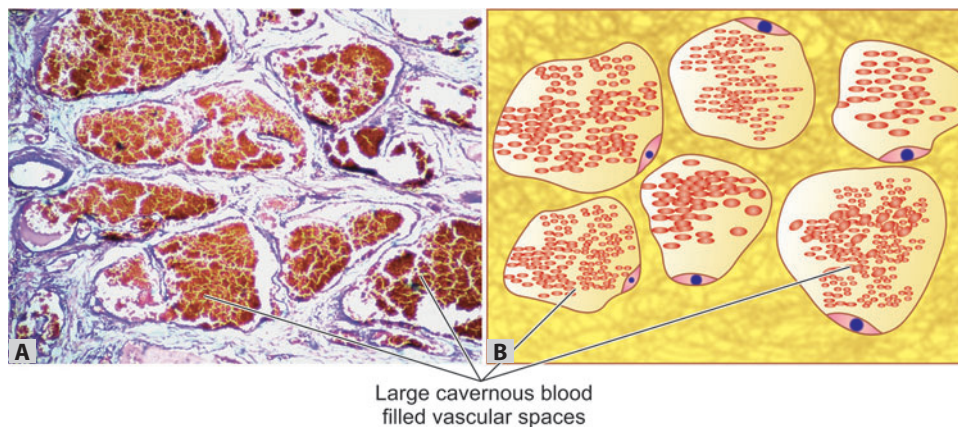
- **Unencapsulated lesion.**
- Composed of **aggregates of closely packed, thin-walled vascular channels**. The size and structure of these **vascular channels resemble normal capillaries**.
- Capillaries are lined by flattened endothelium and their lumen is usually filled with blood. The lumens may contain partially or completely organized thrombus.
- The vessels are separated by scant connective tissue stroma.

Capillary hemangioma:

Aggregates of closely packed thin-walled vascular channels.



Figs 14.11A and B: Microscopic appearance of capillary hemangioma. (A) Photomicrograph; (B) Diagrammatic



Figs 14.12A and B: Microscopic appearance of cavernous hemangioma (A) Photomicrograph; (B) Diagrammatic

Hemangioma: Unencapsulated benign tumor.

Strawberry hemangioma: Type of capillary hemangioma.

Cavernous Hemangioma

- Consists of **large, dilated vascular channels**; compared with small vascular spaces in capillary hemangiomas.

MORPHOLOGY

Gross

- Red-blue, soft, spongy masses and measure 1 to 2 cm in diameter.

- More frequently involve **deep structures**. They are found in the skin, on the mucosal surfaces and visceral organs, such as the **spleen, liver, and pancreas**.

Microscopy (Figs 14.12A and B).

- Consist of sharply defined **unencapsulated** mass.
- Composed of **large, cavernous blood-filled vascular spaces**, separated by a moderate amount of connective tissue stroma. Intravascular thrombosis and dystrophic calcification are common.

Cavernous hemangioma: Large cavernous blood-filled vascular spaces.

Heart Disorders

ISCHEMIC HEART DISEASE

Definition: Ischemic heart disease (IHD) is a group of heart diseases in which there is an **inadequate supply of blood and oxygen to a portion of the myocardium**. Typically, it occurs when there is an imbalance between the demand and supply (perfusion) of oxygenated blood.

List of diseases includes under ischemic heart disease is presented in Box 15.1.

Epidemiology: IHD is the **leading cause of death** in both males and females.

IHD: Results due to imbalance between the coronary blood supply and myocardial demand.

Coronary arteries include:

1. Left anterior descending (LAD)
2. Left circumflex (LCX)
3. Right coronary artery (RCA).

Etiology and Pathogenesis of IHD

Ischemic heart disease is caused by an imbalance between the demand and supply of oxygenated blood to myocardium.

Effects of myocardial ischemia:

1. Insufficient supply of oxygen
2. Decreased supply of nutrients
3. Reduced removal of metabolites.

Decreased/Impaired Coronary Blood Flow

Coronary arterial occlusion is the main cause of myocardial ischemia.

Coronary Atherosclerosis

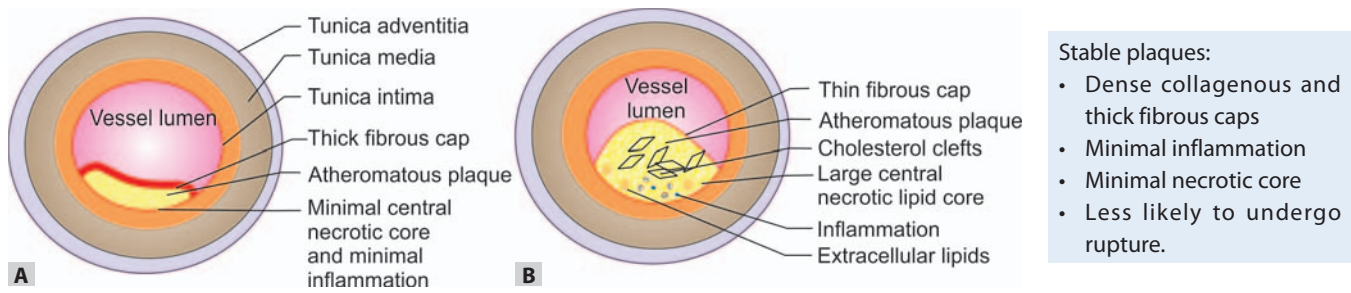
It narrows one or more of the epicardial coronary arteries → decreases the coronary blood flow in about 90% of cases. Hence, **IHD is often known as coronary artery disease (CAD) or coronary heart disease**. **Risk of IHD depends on:** (a) **extent and severity** of pre-existing (fixed) **atherosclerotic occlusion** and (b) **sudden/dynamic morphological changes** in the atheromatous plaque.

Extent and severity of pre-existing (Fixed) atherosclerotic occlusion

- **Number of coronaries affected/involved:** Atherosclerosis may affect **one, two or all three coronaries**.
- **Structure of plaque** (Fig. 15.1): Plaques can be divided into stable and vulnerable (unstable) plaques:
 - **Stable plaques:** They have **dense collagenous and thick fibrous caps** with **minimal inflammation** and negligible underlying atheromatous **necrotic core**. These are **less likely to undergo rupture**.

BOX 15.1: List of ischemic heart diseases

1. Myocardial infarction (ischemia causes the necrosis of heart muscle)
2. Angina pectoris (ischemia is less severe to cause frank necrosis)
 - Stable angina
 - Prinzmetal variant angina
 - Unstable angina
3. Chronic IHD with heart failure
4. Sudden cardiac death due to fatal ventricular arrhythmia



Figs 15.1A and B: Diagrammatic appearance of stable and vulnerable plaque. Cross-section of a vessel with (A) Stable atheromatous plaque; (B) Unstable atheromatous plaque

- **Vulnerable plaque:** They have **core with many foam cells and abundant extracellular lipid**. The **fibrous cap is thin** with few smooth muscle cells or groups of inflammatory cells and **increased inflammation**. These are **likely to undergo rupture**.
- **Degree/extent of stenosis (obstruction) by atheromatous plaques:**
 - **Fixed obstruction less than 70%:** Asymptomatic.
 - **Fixed obstruction of 75% or more:** It results in **critical stenosis** → **precipitates ischemia by exercise** → produces symptom as chest pain—**stable angina**.
 - **Fixed obstruction of 90% and above:** It leads to **inadequate coronary blood flow even at rest—unstable angina**.
- **Rate of development of occlusion:**
 - **Slow progressive occlusion** allows time to **develop collateral circulation to prevent infarction**.
 - **Acute blockage—no time for collateral flow** → **infarction**.
- Factors which influences consequences of coronary atherosclerosis.
 - **Extent of inflammation in atherosclerosis:** Inflammation converts it to vulnerable atheromatous plaque.
 - **Thrombus development on the disrupted plaque.**
 - **Coronary vasoconstriction.**

Sudden Morphological Changes in Atheromatous Plaque

It is known as **acute plaque change** (refer page 394) and is followed by **thrombosis**, produces **unstable angina, myocardial infarction and sudden cardiac death** in most of the patients. Hence, termed **acute coronary syndrome**. This is discussed under pathogenesis of myocardial infarction.

Vulnerable plaque:

- Central necrotic core with many foam cells and abundant extracellular lipid
- Fibrous cap is thin with few smooth muscle cells or groups of inflammatory cells
- Likely to undergo rupture.

IHD: Obstructive atheromatous plaques are predominantly seen within the first several centimeters of the LAD and LCX and along the entire length of the RCA.

Other Causes of Coronary Artery Occlusion (Other Nonatheromatous Causes—refer page 393)

- **Coronary emboli** from thrombi in left side of the heart
- **Coronary vasospasm**
- **Diminished availability of blood or oxygen:**
 - Lowered systemic blood pressure (e.g. shock)
 - Hypoxemia.

Increased Myocardial Demand

It occurs:

- With **myocardial hypertrophy**
- Increased heart rate (**tachycardia**).

ANGINA PECTORIS

Angina pectoris: Three types namely:

1. Stable (typical)
2. Prinzmetal
3. Unstable (crescendo).

Definition: Angina pectoris clinically present as **paroxysmal and recurrent attacks of substernal or precordial chest discomfort** due to **transient myocardial ischemia**, which falls short of inducing necrosis of myocardial cell.

Angina pectoris: Most common manifestation of coronary artery disease (CAD).

Variants of Angina Pectoris

Stable Angina

Stable angina: Most common type of angina pectoris, substernal chest pain induced by exercise.

It is the **most common** and is also known as **typical angina pectoris**.

- **Cause:** **Coronary atherosclerosis** and it develops when myocardial oxygen demand increases with **increased physical activity or emotional excitement**.
- **Pain:**
 - **Site:** **Chest pain in the substernal region**, which may radiate to the left arm, jaw, and epigastrium.
 - **Duration:** It ranges from **1–15 minutes**.
 - **Characteristics:** **Pain is relieved by rest** (which decreases demand) or **by sublingual nitroglycerin**, a strong vasodilator (which increases perfusion).

Prinzmetal Variant Angina

Prinzmetal angina: Due to vasospasm of coronary arteries.

It is an **uncommon atypical form** of angina.

- **Cause:** Due to **spasm of coronary artery having atherosclerosis**. It is **not related to physical activity, heart rate, or blood pressure**.
- **Characteristics:** **Occurs at rest** and responds promptly to vasodilators.

Unstable or Crescendo Angina

Unstable angina:

1. Substernal chest pain at rest
2. Multivessel disease
3. Due to disrupted atheromatous plaque.

- **Cause:** It is caused by the **disruption/rupture of an atherosclerotic plaque** complicated by **thrombosis/embolization/vasospasm/vasoconstriction**.
- **Characteristics:** It is of **prolonged duration and occurs with minimal physical activity or even at rest**.
- **Consequence:** May progress to myocardial infarction and is also referred to as **preinfarction angina**.

MYOCARDIAL INFARCTION

Myocardial infarction: Commonly known as “heart attack” and is the most important form of IHD.

Definition: Myocardial infarction (MI) is a **coagulative type of necrosis of cardiac muscle** and is **due to prolonged severe ischemia**.

Risk Factors

- **Race:** Any race can be affected; whites are affected more than blacks. Indians are also having high-risk of IHD.

- **Age:** Its frequency **risks progressively with age** and peak is between 40 to 65 years of age. It can develop at younger age in patients with major risk factors of atherosclerosis (hyperlipidemia, hypertension, diabetes and cigarette smoking).
- **Sex:** Males have significantly higher risk than females mainly during the reproductive period. However, after menopause the risk is similar to that of males. The protective effect may be due to estrogen.
- **Other risk factors:** Refer under risk factors for atherosclerosis (refer pages 371-3).

Etiology

Q. Discuss etiopathogenesis of myocardial infarction.

Common etiological factor discussed under IHD (refer pages 391-2).

Coronary Atherosclerosis

Coronary artery occlusion in 90% of cases, myocardial infarction is due to atherosclerotic narrowing of one or more coronary arteries.

Nonatheromatous Causes

In about 10% of cases, MI occurs without atherosclerosis of the coronary vessels. It may be due to:

- **Vasospasm without coronary atherosclerosis**
- **Emboli:** The source of which may be:
 - Left atrium in association with atrial fibrillation
 - Left-sided mural thrombus
 - Vegetations of infective endocarditis
 - Intracardiac prosthetic material
 - Paradoxical emboli: The emboli from the right side of the heart or the peripheral veins, which travel through a patent foramen ovale to the coronary arteries.
- **Ischemia due to other causes:**
 - Vasculitis
 - Hematologic disorders like sickle cell disease
 - Amyloid deposition in vascular walls
 - Vascular dissection
 - Lowered systemic blood pressure (e.g. shock).

Pathogenesis of MI

In coronary arteries with pre-existing (fixed) atherosclerotic occlusion, **inadequate coronary perfusion** may occur due to a **new superimposed thrombosis and/or coronary**

vasospasm. Thrombus formation is due to acute plaque change.

Sequence of events in a typical case of MI (Figs 15.2A to E) is as follows:

1. **Acute plaque change:** It is the sudden change/event occurring in an atheromatous plaque. The initial event in pathogenesis of myocardial infarction is sudden change in the atheromatous plaques. These as **acute plaque changes convert partially occlusive atherosclerotic plaque to produce sudden ischemia.** These changes are divided into three categories:
 - **Rupture, fissuring** of plaque → exposes highly thrombogenic plaque constituents → sudden **thrombus formation** → **sudden occlusion of lumen.**
 - **Erosion/ulceration of plaque**
 - ♦ Exposes highly thrombogenic subendothelial basement membrane
 - ♦ Sudden **thrombus formation**
 - ♦ **Sudden occlusion of lumen.**
 - **Hemorrhage into the central core of plaque** → increases the plaque size → sudden **occlusion of lumen.**

Factors that trigger acute plaque change:

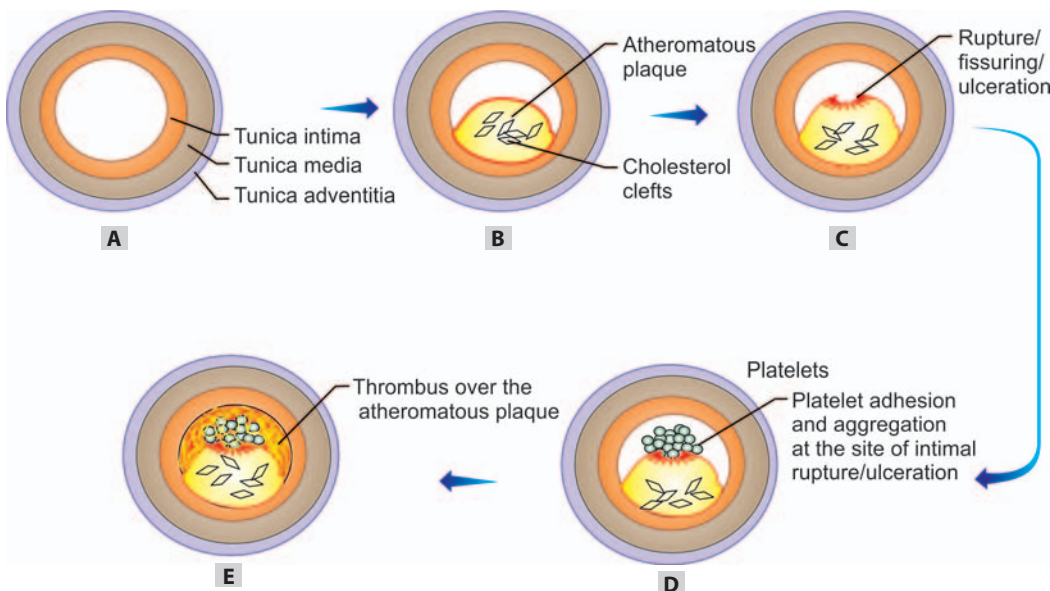
- **Intrinsic:** Plaque composition and structure (namely **vulnerable plaque**).
- **Extrinsic:** Blood pressure and platelet reactivity which induces total thrombotic occlusion of

already narrowed coronary artery by atheromatous plaque.

2. **Formation of microthrombi:** Acute plaque changes exposes thrombogenic subendothelial collagen → platelets adhere to the site → platelet activation and aggregation → formation of microthrombi on the atheromatous plaque → **partial or complete occlusion of the affected coronary artery.**
3. **Vasospasm:** Activated platelets, endothelial cell and inflammatory cells release mediators → cause **vasospasm** at the sites of atheroma → further narrowing of the lumen.
4. **Activation of the coagulation pathway:** Tissue factor released at the site of acute plaque change → activates coagulation system → **increase the size of the thrombus.**
5. **Complete occlusion of vessel:** Within minutes, the thrombus may completely occlude the lumen of the vessel.
6. **Myocardial necrosis:** Complete occlusion → results in **ischemic coagulative necrosis of the area supplied** by the particular coronary artery. The anatomic area supplied by that artery is called as the area at risk.

Acute plaque change: Sudden morphological changes occurring in an atheromatous plaque. These include:

1. Rupture and fissuring
2. Erosion and ulceration
3. Hemorrhage into central core of plaque.



Myocardial infarction: Initial event in the blockage of the coronary is due to a thrombus developing over the ruptured atheromatous plaque

Figs 15.2A to E: Sequential changes in coronary artery atherosclerosis causing occlusion of lumen in ischemic heart disease: (A) Normal coronary artery; (B) Atherosclerosis of coronary artery; (C) Acute plaque change; (D) Platelet adhesion and aggregation at the site of plaque disruption; (E) Formation of occlusive thrombus

Consequence of Myocardial Ischemia

These include functional, biochemical and morphological changes. Morphological changes can be divided into reversible and irreversible damage/injury.

- A. **Reversible injury:** These changes are potentially reversible and include:
- **Biochemical changes:** Cessation of aerobic glycolysis occurs within seconds of myocardial ischemia → **decreased production of ATP** (adenosine triphosphate) → **accumulation of potentially toxic metabolites** (such as lactic acid).
 - **Functional disturbances:** Loss of contractility within 60 seconds → can precipitate acute heart failure.
 - **Morphological changes:** They are seen at ultrastructural level such as **mitochondrial swelling, glycogen depletion and myofibrillar relaxation**. They also develop within a few minutes.
- B. **Irreversible injury:** It develops only after prolonged, severe myocardial ischemia of more than 20–40 minutes.
- **Biochemical changes:** They cause **leakage of cytoplasmic proteins into the blood**. In the early phases of myocardial cell necrosis, there is breakdown of the sarcolemmal membrane → leakage of intracellular proteins (such as **myoglobin, LDH, CK, and troponins I and T**) into the blood. The levels of these leaked myocardial proteins in the blood is **used for the diagnosis as well as management of MI**.
 - **Functional disturbances:** Arrhythmias.
 - **Morphological changes:** **Coagulative necrosis** of cardiac muscle fibers usually complete within 6 hours of the onset of myocardial ischemia.
- Zones damaged:** First necrosis in the subendocardial zone → later transmural infarct.

Classification of Myocardial Infarct

Q. Classify myocardial infarction.

- A. **Depending on the thickness of myocardium involved:**
- **Transmural infarct:**
 - Ischemic necrosis of the **entire thickness of the ventricular wall**. However, a narrow rim (approximately 0.1 mm) of subendocardial myocardium is preserved due to diffusion of oxygen and nutrients from the ventricular lumen.
 - Most myocardial infarcts are transmural.
 - Usually **associated with chronic coronary atherosclerosis, acute plaque change, and superimposed thrombosis**.

- **Subendocardial (nontransmural) infarct:**
 - Ischemic necrosis of **inner one-third to one-half of the ventricular wall**.
 - Occurs due to **plaque disruption** followed by a **coronary thrombus, which undergoes lysis** or prolonged, severe reduction in systemic blood pressure. For example, shock superimposed on chronic, coronary stenosis.
 - **Multifocal microinfarcts:** Develop with occlusion of small vessel (e.g. vasculitis, embolization) and may not show any changes in ECG.
- B. **Depending on the age of the infarct: Recent (fresh) or old (healed).**
- C. **Depending on the anatomic region involved:** Anterior, posterior, lateral, septal and their combination like posterolateral.
- D. **Depending on the electrocardiographic changes:**
- ST elevation myocardial infarct (**STEMI**) found in transmural infarct,
 - Non-ST elevation infarct (**NSTEMI**) found in subendocardial infarct and
 - Electrocardiographically silent with nonspecific changes in microinfarctions (depending on the extent and location of the vascular involvement).

Differences between transmural and subendocardial infarct are listed in Table 15.1.

Myocardial infarction types:

1. Transmural
2. Subendocardial
3. Microscopic.

TABLE 15.1: Differences between transmural and subendocardial infarct

Characteristics	Transmural infarcts	Subendocardial infarcts
Nature of lesion	Unifocal and solid	Multifocal and patchy
Distribution	Specific coronary artery	Circumferential
Thrombus in the coronary artery	Common	Rare
Shock	Often causes shock	Often result from hypotension or shock
Pericarditis	Common	Absent
Cardiac aneurysm	May develop	Does not develop.
ECG changes	Elevation of ST segment → ST elevation infarcts (STEMIs)	Non-ST elevation infarcts

MORPHOLOGY**Q. Describe the sequential changes seen in myocardial infarction.****General features:**

- Most of transmural infarcts (>50%) involve at least a portion of the left ventricle and/or interventricular septum.
- ~ 15–30% of MI, which involve posterior or posteroseptal wall also extend to involve right ventricle.
- Isolated infarction of the right ventricle and infarction of the atria are rare.

Pattern of left ventricular infarcts (Fig. 15.3):

- **Left anterior descending (LAD) coronary artery occlusion (40–50%):** Infarcts involve:
 - Anterior wall of left ventricle near the apex
 - Anterior portion of ventricular septum
 - Apex circumferentially.
- **Right coronary artery occlusion (30–40%):** Infarcts involves:
 - Region of the inferior/posterior wall of left ventricle
 - Posterior portion of interventricular septum (inferior infarct)
 - Inferior/posterior right ventricular free wall (in some).
- **Left circumflex coronary artery occlusion (15–20%):** Infarcts involve the lateral wall of left ventricle except at the apex.

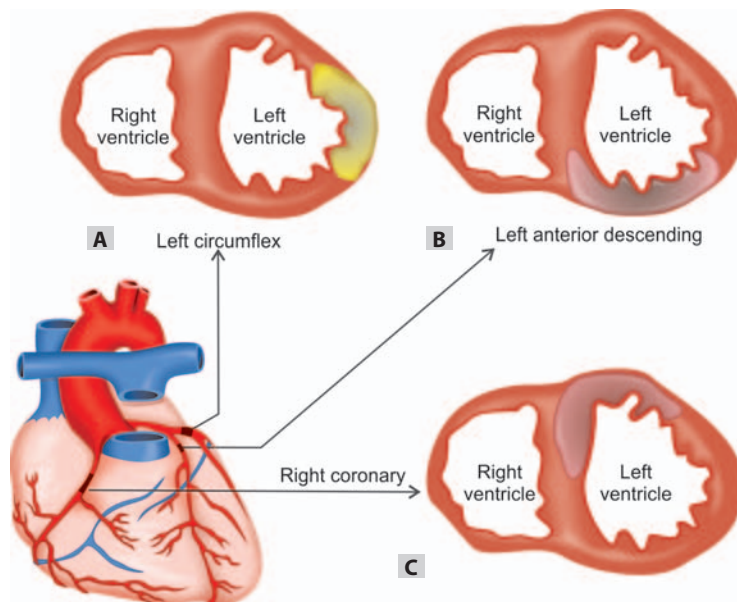
The sequences of the morphologic changes in acute MI and subsequent healing are summarized in Table 15.2.

Gross (Table 15.2)

MI: Gross and microscopic changes develop only hours to days after the onset of ischemia.

- **Within first 12 hours:** No identifiable/apparent gross changes are seen. **Triphenyl tetrazolium chloride (a histochemical stain)** can grossly identify infarct within 2–3 hours after onset.
 - Noninfarcted myocardium appears **brick-red** (lactate dehydrogenase activity is preserved).
 - Infarcted area remains **unstained pale** (loss of dehydrogenases).
 - Old infarcts appear **white and glistening**.
- **By 12–24 hours (Fig. 15.4):** Grossly identifiable. Appears **pale reddish-blue area** (due to stagnated, trapped blood) → progressively becomes **sharply defined, yellow-tan, and soft**.
- **After 3–5 days:** Mottled with a **central pale, yellowish, necrotic region** with well-demarcated border of **hyperemic zone** (due to granulation tissue).
- **By 10 days to 2 weeks:** Appears **soft and rimmed by a hyperemic zone** of highly vascularized granulation tissue.
- **After 2 weeks (Fig. 15.4):** Older, healed infarcts appear **firm, pale gray and contracted** → develops into a **fibrous scar** (Fig. 15.4).

MI: Characterized by coagulative necrosis within 24 hours.

**Triphenyltetrazolium chloride:**

Histochemical stain that can grossly identify infarct within 2–3 hours after onset.

MI: Coagulative necrosis occurs only 20–40 minutes after the ischemia.

Figs 15.3A to C: Pattern of left ventricular infarcts resulting from occlusion of each of the three main coronary arteries. (A) Posterolateral infarct develops following occlusion of the left circumflex artery; (B) Anterior infarct develops following occlusion of the anterior descending branch of left coronary. The infarct is located in the anterior wall of left ventricle and adjacent two-thirds of the interventricular (AV) septum; (C) Posterior infarct results from occlusion of the right coronary artery and involves the posterior wall, including the posterior third of the AV septum

Q. Describe the morphological changes occurring in myocardial infarction.

TABLE 15.2: Sequence of morphologic changes in myocardial infarction

Duration	Gross changes	Light microscopic changes
Reversible injury		
0–½ hour	None	None (electron microscope—relaxation of myofibrils; loss of glycogen and swelling of mitochondria)
Irreversible injury		
½–6 hours	None	Usually no changes but may show waviness of fibers at border (electron microscopy; mitochondrial amorphous densities)
6–12 hours	Dark mottling	Early coagulative necrosis
12–24 hours	Dark mottling	Coagulative necrosis; pyknotic nuclei; increased eosinophilia of cytoplasm; contraction band necrosis at margins; beginning of neutrophilic infiltrate
1–3 days	Mottling with yellow-tan center	Coagulation necrosis, loss of nuclei and striations; increased interstitial infiltration of neutrophils
3–7 days	Hyperemic border; central yellow-tan	Disintegration of dead myocardial cells, with disintegrating neutrophils; phagocytosis of dead cells by macrophages at the border of infarct
7–10 days	Maximally yellow-tan and soft	Phagocytosis of dead cells; formation of granulation tissue at margins
10–14 days	Red-gray depressed infarct borders	Well-established granulation tissue and collagen deposition
2–8 weeks	Gray-white scar, progressive from border toward the center of the infarct	Increased collagen deposition and decreased cellularity
>2 months	Scarring complete	Dense collagenous scar tissue

Reperfusion and its Effects on MI

The ischemic myocardium can be rescued by restoring the myocardial blood flow as quickly as possible → by reperfusion (refer page 15-16).

- **Methods of reperfusion:** It may be achieved by dissolving the thrombus using thrombolytic drugs or angioplasty/stent placement, or coronary artery bypass graft (CABG) surgery).
- **Benefits and outcome of reperfusion:** It depends on rapidity of restoration. Reperfusion within 20 minutes of the onset of ischemia may completely prevent necrosis.

MORPHOLOGY OF REPERFUSED INFARCT

- **Gross:** Reperused infarcted area is **hemorrhagic** because of the microvasculature is injured during ischemia and leaks with restoration of blood flow.
- **Microscopy:**
 - **Accelerates acute inflammatory responses:** Neutrophils accumulate more rapidly but also disappear more rapidly.
 - **Contraction band necrosis:** It is **one of the characteristic feature**. They appear as thick, irregular, transverse, intensely eosinophilic intracellular bands in necrotic myocardial cells.

Reperfusion injury is due to:

1. Reactive oxygen species
2. Inflammatory cells
3. Calcium overload.

Complication of Reperfusion

Reperfusion injury is mediated by **reactive oxygen species, calcium overload, and severe inflammatory reaction during reperfusion** (refer page no 15-16).

Diagnosis of Myocardial Infraction

It is based on (1) clinical symptoms, (2) electrocardiographic changes and (3) laboratory findings.

Diagnosis of MI:

1. Clinical symptoms
2. Electrocardiographic changes
3. Measurement of serum CK-MB and cardiac troponins.

Clinical Symptoms

1. **Severe, retrosternal chest pain** that can radiate to neck, jaw, epigastrium or left arm.

MICROSCOPY (FIG. 15.5 AND TABLE 15.2)

- **First 6 hours:** Earliest changes can be detected only by electron microscopy (ultrastructural changes)
 - **Reversible injury (0–1½ hours):** Relaxation of myofibrils, loss of glycogen, and mitochondrial swelling.
 - **Irreversible injury (½–6 hours):** Develops after 30–60 minutes of ischemia. The changes include **mitochondrial amorphous matrix densities, focal disruption of sarcolemma and clumping and margination of nuclear chromatin.**
- **6–12 hours:** Coagulative necrosis begins and shows edema and hemorrhage. Other changes include:
 - **Wavy fibers:** They represent noncontractile, stretched, buckled dead myofibrils at the periphery of the infarct.
 - **Vacuolization of myocardial cell (myocytolysis):** It is characterized by large vacuolar spaces within myocardial cell (probably water).
- **12–24 hours** (Figs 15.5B and F): Characteristic changes of **coagulation necrosis** appear. These include: (1) **preserved cell outlines of myocardial cell**, (2) **deeply eosinophilic cytoplasm** and (3) **pyknotic nuclei**.
- **1–2 days:**
 - **Acute inflammatory reaction** characterized by accumulation of polymorphonuclear leukocytes (Fig. 15.5C) **at the periphery/borders of infarct.**
 - **Continuation of coagulation necrosis:** Nuclei disappear, and striations become less prominent. Margins show contraction band necrosis.
- **3–7 days:**
 - **Appearance of macrophages:** The polymorphonuclear leukocytes are replaced by macrophages. **Macrophages phagocytose** and remove the necrotic myocardial cells and neutrophil fragments at the border of infarct.
 - **Granulation tissue:** Appears **at the periphery of infarct**, and gradually extends toward the center.
- **1–2 weeks:** **Process of healing starts from its margins** toward its center which is responsible for most advanced healing at the periphery.
 - **Well-established highly vascularized granulation tissue**, which consists of proliferating capillaries and fibroblasts.
 - **Fibroblasts proliferate** and collagen deposition proceeds.
- **2–8 weeks** (Fig. 15.5D): **Increased collagen deposition and less cellularity.**
- **>2 months** (Fig. 15.5E): Dense collagenous scar. In a completely healed infarct, it is not possible to determine its age.

2. Rapid, weak pulse.
3. Profuse sweating (diaphoresis).
4. Dyspnea due to impaired contraction of the ischemic myocardium and the resultant pulmonary edema.
5. About 10–15% of cases it may be entirely asymptomatic (“silent” MIs) common in elderly and in patients with diabetes mellitus.

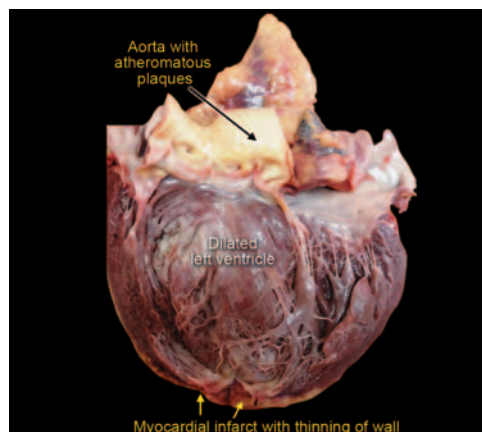


Fig. 15.4: Myocardial infarct at the apex of heart

Electrocardiographic Changes

ECG changes in myocardial infarction: Inverted T waves, elevated ST segments, Q waves.

1. Q waves.
2. ST segment changes.
3. T wave inversions.

Laboratory Findings

Q. Laboratory diagnosis of myocardial infarction.

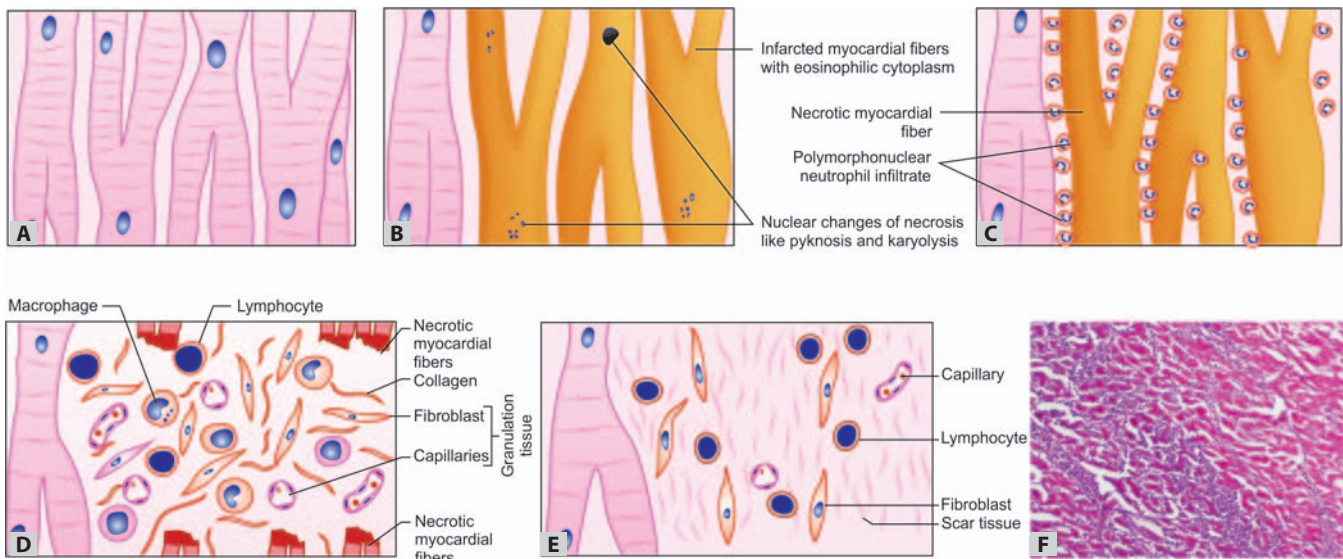
These findings are mainly, due to leakage of proteins from the necrotic myocardial cells into the blood circulation:

1. **Cardiac troponins:** These are proteins involved in heart muscle contraction. **Increased plasma levels establish the diagnosis of myocardial infarction.**
 - **Cardiac-specific proteins are of two types: Troponins I (TnI) and T (TnT).** They are **most sensitive and specific markers** of myocardial infarction. Levels begin to rise at 2–4 hours and **peaks at 48 hours**. The elevated troponin levels may **remain for 7–10 days** after acute MI.

Cardiac troponin:

1. Gold standard for diagnosis of acute MI
2. Most sensitive
3. Most specific for MI.

2. **Cardiac creatine phosphokinase (CK):** It is a **non-specific enzyme marker** and it is present in brain, myocardium, and skeletal muscle. It has two isoforms designated “M” and “B”. **MB heterodimers chiefly in cardiac muscle** (lesser amounts in skeletal muscle).



Figs 15.5A to F: Microscopic changes observed during development of a myocardial infarct. (A) Normal myocardium; (B) After about 12–18 hours, the infarcted myocardium shows myocardial fibers with eosinophilia; (C) About 24 hours after the onset of infarction, polymorphonuclear neutrophils infiltrate at the periphery of infarcted area; (D) After about 3 weeks, the infarcted area consists of granulation tissue infiltrated by lymphocytes and macrophages. (E) After 3 months or more, the infarcted region is replaced by collagenous tissue; (F) Photomicrograph of acute myocardial infarction showing coagulative necrosis with acute inflammatory infiltrate

EM changes with reversible injury:

- Relaxation of myofibrils
- Loss of glycogen
- Mitochondrial swelling.

EM changes with irreversible injury:

- Mitochondria with amorphous matrix density
- Focal disruption of sarcolemma
- Clumping and margination of nuclear chromatin.

Neutrophils: Present between 48–72 hours.

Macrophages: Predominant cells after 72 hours and phagocytose dead cells.

- **MB form of creatine kinase (CK-MB) is sensitive but not specific**, because it is also raised with skeletal muscle injury.
- CK-MB levels **rise within 2–4 hours** of the onset of MI, **peaks at 24 hours**, and returns to **normal within 72 hours**.

Myoglobin: Earliest enzyme to increase after MI.

Reinfarction: CK-MB reappears after 3 days.

Various laboratory markers of MI are presented in Table 15.3 and Figure 15.6.

MI: If CK-MB and troponin remains unchanged for a period of 2 days, diagnosis of MI can be excluded.

CK-MB: Enzyme of choice for diagnosing reinfarction.

3. **Lactate dehydrogenase (LDH): It not specific marker.** It starts rising after 24–48 hours. It remains for many days and returns to normal in 7–14 days.
4. **Myoglobin:** It is an oxygen-carrying respiratory protein found only in skeletal and cardiac muscle. It is an **earliest marker of MI**, the level rises within 1–3 hours, **peaks in about 8–12 hours** and return to normal in about 24–36 hours.

Complications of Myocardial Infarction

Q. Mention the complications of myocardial infarction.

The risk of complications depends on: (1) infarct size, (2) location, and (3) thickness of myocardium involved (subendocardial or transmural).

1. **Left ventricular failure and cardiogenic shock:** MI produces contractile functional abnormalities of left ventricle and is roughly proportional to the size of infarct.

- **Mild dysfunction:** → left ventricular failure → pulmonary edema.

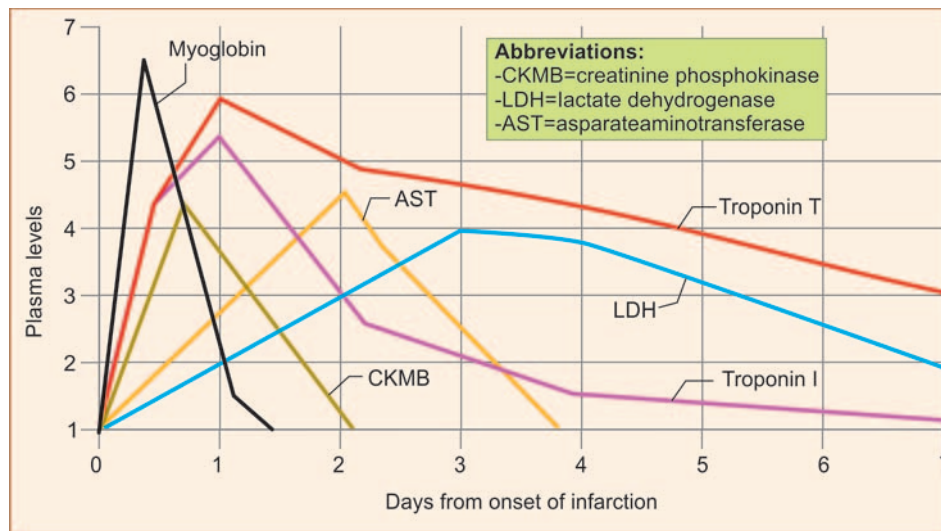


Fig. 15.6: Various enzyme levels in myocardial infarction

TABLE 15.3: Laboratory markers of myocardial infarction`

Marker	Onset	Peak	Normalization	Significance
Troponins T & I	2–4 hours	48 hours	7–10 days	Most sensitive and specific marker
CK-MB	2–4 hours	24 hours	72 hours	Sensitive but not specific
LDH	24–48 hours	Many days	7–14 days	Not specific marker
Myoglobin	1–3 hours	8–12 hours	24–36 hours	Earliest marker

- **Cardiogenic shock:** Severe “pump failure” occurs with a **large infarct** (>40% of the left ventricle) → cardiogenic shock → **fatal**.

MI: Ventricular fibrillation is the most common cause of death.

2. **Arrhythmias:** Almost most MI patients develop **abnormal cardiac rhythm** → myocardial irritability and/or conduction disturbances → arrhythmias. These include **sinus bradycardia, tachycardia, ventricular tachycardia, and ventricular fibrillation**. In inferoseptal infarcts → **partial or complete heart block**.

MI: Heart is maximally soft during 3–7 days; risk of myocardial rupture more during this period.

3. **Myocardial rupture:** Necrosis and neutrophilic infiltration → causes softening and weakening of myocardium → lead to cardiac rupture. **Most frequent during 3–7 days after transmural infarcts**.
 - **Rupture of the ventricular free wall** (Fig. 15.7): It is most common → result in **hemopericardium and cardiac tamponade**.
 - **Rupture of the ventricular septum** (Fig. 15.7): It is less common → lead to an **acute VSD and left-to-right shunt**.

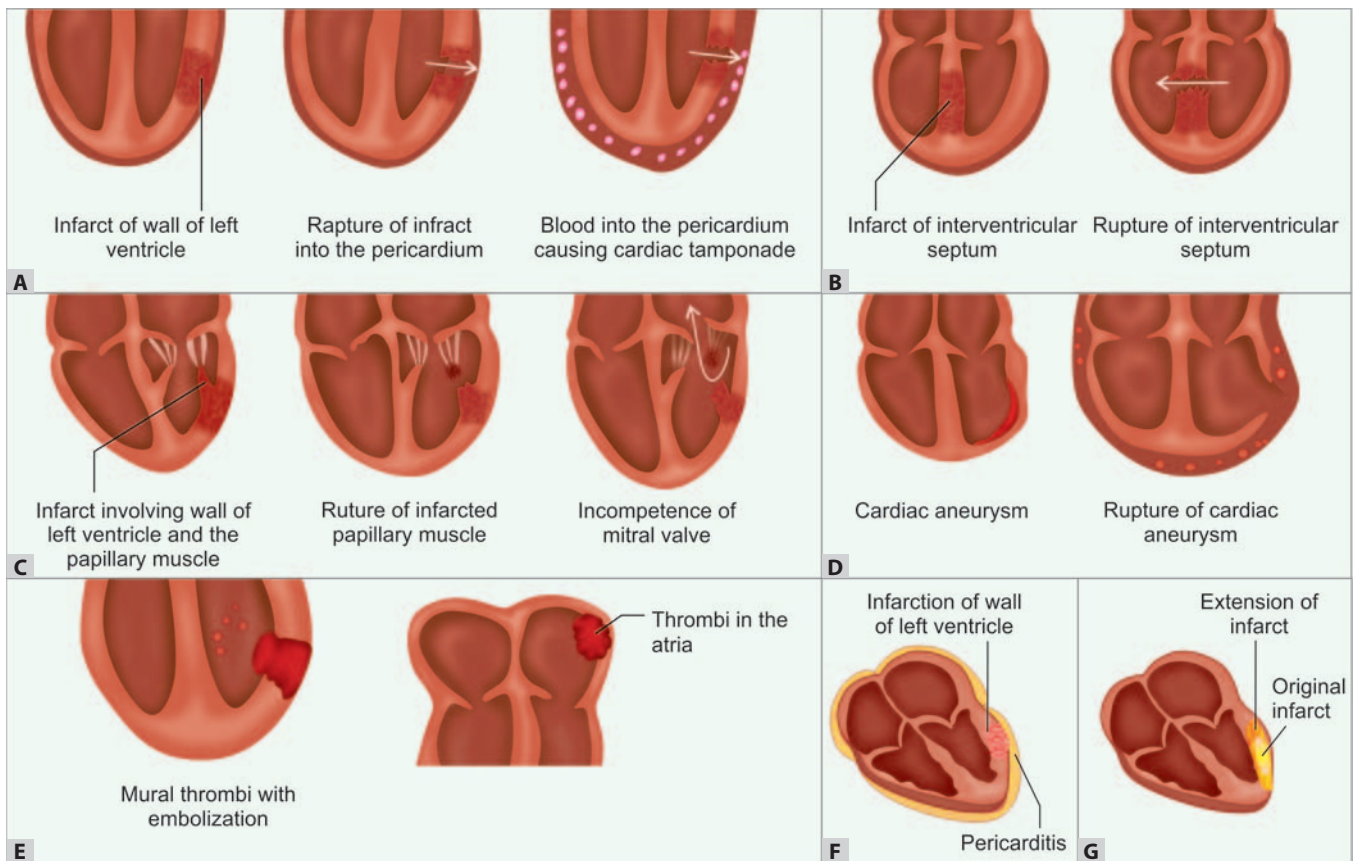
- **Rupture of papillary muscle** (Fig. 15.7): It is least common → leads to acute severe mitral regurgitation.

Posteromedial papillary muscle rupture:

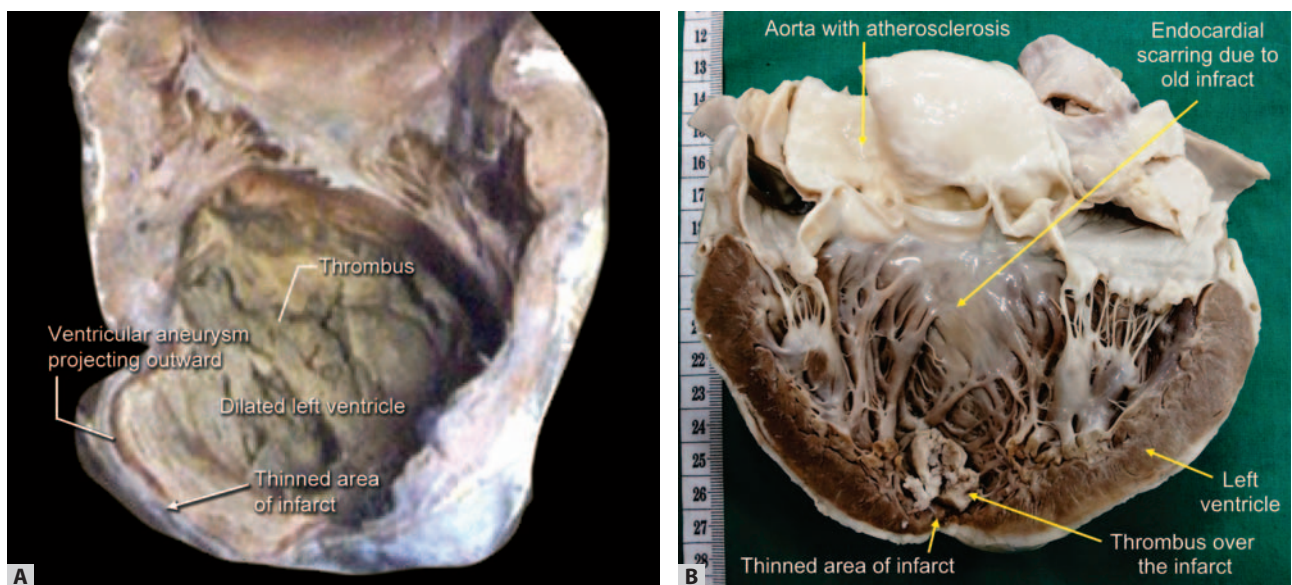
1. Due to thrombosis of RCA
2. Causes mitral regurgitation.

4. **Dilatation of ventricular chamber:** Area of infarct being weak region may be disproportionately stretched → dilation of the infarct region (especially with anteroseptal infarcts) not severe enough to cause aneurysm described below.
5. **Ventricular aneurysm** (Figs 15.7 and 15.8A): After acute transmural infarction, the affected ventricular wall may bulge outward during systole resulting ventricular aneurysm. It develops as a late complication of large transmural infarcts.
6. **Mural thrombus** (Figs 15.7 and 15.8B): Infarct → causes local abnormality in myocardial contractility (causing stasis) and endocardial damage (creating a thrombogenic surface) → favor **mural thrombosis** → left-sided **thromboembolism**. They may also develop **within ventricular aneurysms**.

MI: Mural thrombus on the infarcted site is likely source of emboli.



Figs 15.7A to G: Various complications of myocardial infarction



Figs 15.8A and B: (A) MI with ventricular aneurysm containing thrombus; (B) Myocardial infarct with a thrombus over the infarcted area

7. **Pericarditis:**

Pericarditis in MI:

1. Early—due to acute inflammation
2. Late—autoimmune mechanism.

- **Early pericarditis:** A transmural myocardial infarct → can involve the pericardium → cause **fibrinous or fibrinohemorrhagic** pericarditis (Fig. 15.7). Usually develops on **second or third day**.
- **Delayed form of pericarditis (postmyocardial infarction syndrome/Dressler syndrome):** Develops 2 to 10 weeks after infarction—**probably immunologically mediated**.

8. **Extension of infarct:**

- New areas of repeated necrosis can occur adjacent to an existing myocardial infarct causing extension of infarct.
- In extended infarct, healing is more advanced in the central zone than the periphery of the infarct. This is in contrast with that simple infarct, in which the healing is more advanced at the periphery.
- **Cause of extension:** It may be due to retrograde propagation of a thrombus or proximal vasospasm, microemboli, or an arrhythmia.

9. **Infarction of right ventricle:** It is **unusual**. However, part of right ventricular myocardium may be involved with infarction of adjacent posterior left ventricle and ventricular septum → venous circulation pooling → systemic hypotension.

10. **Progressive late heart failure (chronic IHD):** Usually develops due to the functional decompensation of hypertrophied noninfarcted myocardium.

Complications of myocardial infarction depending on the duration are listed in Table 15.4.

MI is the leading cause of death in diabetes mellitus.

Silent MI in diabetes mellitus.

Complications of MI

1. Left ventricular failure and cardiogenic shock
2. Arrhythmias
3. Myocardial rupture
4. Dilatation of ventricular chamber
5. Ventricular aneurysm
6. Mural thrombus
7. Pericarditis
8. Extension of infarct
9. Infarction of right ventricle
10. Progressive late heart failure.

TABLE 15.4: Complications of myocardial infarction depending on the duration

Duration	Complications
First 72 hours	<ul style="list-style-type: none"> • Cardiogenic shock • Conductive disturbances—arrhythmias • Acute pulmonary edema (left ventricular failure) • Rupture external-hemopericardium (cardiac tamponade)
First 3 weeks	<ul style="list-style-type: none"> • Any of the above • Pericardial effusion • Rupture: (1) myocardial, (2) papillary muscle, (3) chordae tendineae and (4) interventricular septum • Thromboembolism
Late complications	<ul style="list-style-type: none"> • Repeat infarction • Cardiac aneurysm • Chronic ischemic heart disease (ischemic cardiomyopathy) • Congestive heart failure • Pulmonary hypertension • Autoimmunity-delayed form of pericarditis • Chronic ischemic heart disease (IHD)

Chronic Ischemic Heart Disease

Progressive heart failure developing as a consequence of ischemic damage myocardium is termed as chronic IHD.

Sudden Cardiac Death

Sudden cardiac death: Unexpected death occurring within one hour after the symptoms.

Definition: Sudden cardiac death (SCD) is an unexpected death from cardiac causes in individuals without symptomatic heart disease or within 1 hour of the onset of symptoms.

Sudden cardiac death: Most commonly due to ventricular fibrillation.

Cause: Usually due to fatal arrhythmia (e.g. asystole, ventricular fibrillation) occurring in IHD. **Acute myocardial ischemia is the most common cause for fatal arrhythmias.** Usually associated with coronary atherosclerosis.

INFECTIVE ENDOCARDITIS

Q. Describe heart in infective endocarditis.

Definition: Infective endocarditis (IE) is defined as an **infection, usually bacterial, of the endocardium of heart**

valves or the mural endocardium. It results in focal area of colonization or invasion by microbial agents.

- **Sites of colonization by microbial agents:**
 - Heart valves
 - Mural endocardium
 - Congenital defect (e.g. patent ductus arteriosus or coarctation of the aorta)
 - Other less common sites: (i) aorta, (ii) aneurysmal sacs, (iii) other blood vessels, and (iv) prosthetic devices.
- **Lesions produced:**
 - **Vegetations:** These are lesions seen at the site of colonization or invasion. Composed of thrombotic debris and organisms.
 - **Destruction** of the underlying cardiac tissues/site of colonization is often observed.
- **Microbes responsible:**
 - **Bacterial infections** (bacterial endocarditis) in most of the cases.
 - **Other microbes:** Fungi, *Chlamydia*, *Rickettsiae* and microorganisms.

Classification

A. **According to the clinical course** (Table 15.5): **Acute or subacute** endocarditis. The clinical course depends

TABLE 15.5: Differences between acute and subacute endocarditis

Characteristic	Acute infective endocarditis	Subacute infective endocarditis
Onset	Acute	Insidious
Condition of valve	Infection of normal heart/cardiac valve	Infection of structurally abnormal/deformed valves
Virulence of organisms	Highly virulent (suppurative)	Low virulent
Lesions	Affected valve is rapidly destroyed	Less destructive
Clinical features	Clinical features of acute infection	Clinical features of complications
Course	Death within 6 weeks	Protracted course of weeks
Complications	Acute heart failure or overwhelming sepsis	Infectious complications are uncommon

on the virulence of the infecting microorganism and whether any underlying cardiac disease is present.

- **Acute infective endocarditis:** The characteristic features are:
 - **Acute** in onset
 - Infection of **normal heart/cardiac valve**
 - **Caused by highly virulent (suppurative) organism.** Mainly by *Staphylococcus aureus* and *S. pyogenes*.
 - **Affected valve is rapidly destroyed** by necrotizing, ulcerative valvular lesions.
 - **Death within 6 weeks in many patients**, due to complications like acute heart failure or overwhelming sepsis.
- **Subacute infective endocarditis:** The characteristic features are:
 - **Insidious** in onset.
 - It **involves structurally abnormal/deformed valves** (e.g. rheumatic heart disease or congenital heart disease).
 - **Caused by organisms are of lower virulence** (e.g. *Streptococcus viridans* or *Staphylococcus epidermidis*).
 - The lesions are **less destructive**.
 - Pursue a **protracted course of weeks to months** and typically survive for 6 months or more. Mostly cured with antibiotics.
 - Infectious **complications are uncommon**.

B. **According to anatomic location and causative microbes:** The clinical presentations described above are unusual today because of advance in antimicrobial therapy. So, IE is now classified according to the anatomical location and the offending organism, e.g. infective endocarditis of mitral valve due to *Staphylococcus aureus*.

Infective endocarditis: Acute type can cause destruction of normal valves.

Infective endocarditis: Subacute type is minimally destructive and usually involves previously abnormal/damaged valves.

Infective endocarditis: Prompt diagnosis and effective treatment is important.

Etiology

Q. Describe the etiology and pathogenesis of infective endocarditis.

Causative Organism

Many organisms can cause endocarditis, most originate from the normal flora of the body surfaces, and enter into

the bloodstream in a variety of different ways. More than 90% of IE is caused by streptococci and staphylococci.

- ***Staphylococcus aureus***: It is **virulent organisms** commonly found on the skin. It can infect either **healthy or deformed** valves. It is the major organism responsible for IE in **intravenous drug abusers**.

Staphylococcus aureus: Most common pathogen causing IE in IV drug addicts.

- ***Streptococcus viridans***: It is present in the **normal flora of the oral cavity**. It is the most commonly causes endocarditis of previously **damaged or abnormal valves**.

Streptococcus viridans: Most common cause of IE of previously damaged or abnormal valves.

- **Coagulase-negative staphylococci** (e.g. *S. epidermidis*): It is the most common cause for **prosthetic valve** endocarditis.
- **Other bacteria** commensals in the oral cavity:
 - HACEK group (*Haemophilus*, *Actinobacillus*, *Cardiobacterium*, *Eikenella*, and *Kingella*)
 - Enterococci.
- **Other agents**: It includes gram-negative bacilli and fungi.
- **Culture-negative endocarditis**: They constitutes 10–15% of cases in which no organism can be isolated from the blood.

Predisposing Factors

Three main factors which may predispose to IE are: (1) conditions that lead to bacteremia, (2) underlying heart disease, and (3) impaired host defense mechanism.

1. **Conditions with bacteremia**: Most important predisposing factors to the development of endocarditis are conditions that lead to bacteremia.

Sources of microbes: **Transient bacteremia** from any cause may lead to infective endocarditis.

Infective endocarditis: Conditions which can produce transient bacteremia include:

1. Obvious infection elsewhere
 2. Dental or surgical procedure
 3. Urinary catheterization
 4. Obstetric procedures
 5. Contaminated needle shared by intravenous drug users
 6. Trivial breaks in the epithelial barriers of the gut, oral cavity, or skin.
2. **Underlying heart disease**: IE may involve either normal or damaged heart/valve. IE on previously normal valves develop usually with high virulent organisms. Underlying heart disease is one of the predisposing factors for IE. These diseases include:

- **Congenital heart disease**: Unrepaired and repaired congenital defects—most common predisposing condition (e.g. valvular or septal defect).
- **Mitral valve prolapse** (MVP) in adults.
- **Chronic rheumatic heart disease** (mitral valve in over 85% of cases, and the aortic valve is involved in 50%).

Underlying heart disease predisposing to IE:

1. Congenital heart disease
2. Chronic rheumatic heart disease
3. Mitral valve prolapse.

- **Degenerative calcific valvular diseases** (mainly in elderly).
- Bicuspid aortic valve.
- **Iatrogenic**
 - Artificial (prosthetic) valves
 - Indwelling vascular catheters.

3. **Impaired host defense mechanism**: It may occur in:
 - Diabetes mellitus
 - Malignancies: Leukemia and lymphomas
 - Cytotoxic therapy
 - Neutropenia.

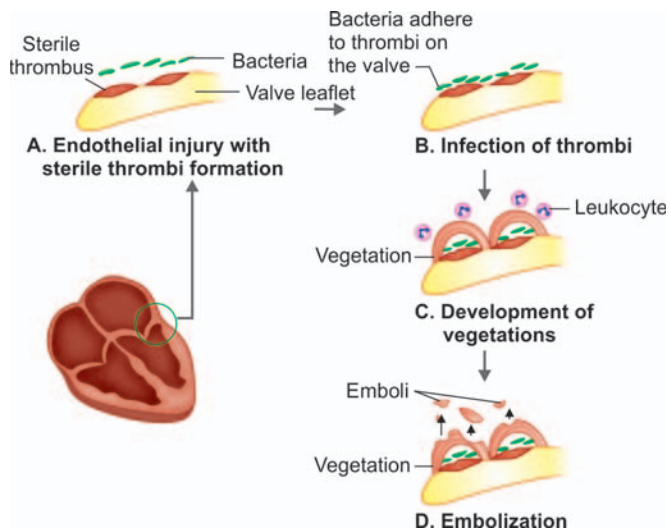
Prevention of IE in patients with predisposing factors: Prophylaxis with antibiotics.

Pathogenesis

Mechanism by which virulent organisms infect apparently normal valves is poorly understood.

The probable sequence of events (Figs 15.9A to D), which occur with the infection of a damaged valve by less-virulent organisms is as follows:

1. **Endocardial damage and denudation**: Development of the infective endocarditis follows a predictable sequence. Damaged valve or a congenital defect creates abnormal high-velocity blood flow at the center and turbulent flow at the periphery → cause endocardial damage and denudation of endothelial surfaces.
2. **Formation of sterile thrombus** (Fig. 15.9A): Damaged endothelial surface → attracts focal deposition of platelets and fibrin → aggregation of platelets and fibrin → formation of small sterile vegetations (sterile thrombi).
3. **Adherence of the microorganisms** (Fig. 15.9B): Transient bacteremia → microorganisms gain access to the circulation → adherence deposition to the sterile vegetations (infection of thrombi).
4. **Proliferation of microorganisms within vegetations** (Fig. 15.9C): On the deposited microorganisms, further



Figs 15.9A to D: Pathogenesis of vegetation on a valve leaflet in infective endocarditis. (A) Focal endothelial injury of a valve leaflet is covered with tiny deposits of platelets and fibrin to form a thrombus; (B) Circulating microorganisms colonize the platelet thrombus; (C) Further platelets and fibrin are laid down and the microorganisms proliferate slowly; (D) Vegetations fragment and form emboli

platelets and fibrin are laid down, which separates them from the bloodstream. Microorganisms proliferate within the vegetations and form colonies.

5. **Formation of emboli (Fig. 15.9D):** The vegetation may get detached and form infective emboli → cause spread of infection to visceral organs and brain.

Infective endocarditis in intravenous drug abusers: Pathogenic organisms gain entry through skin along with the drugs, with *S. aureus* causing more than 50% of the infections involve tricuspid valves.

Prosthetic valve endocarditis:

1. Most commonly affects aortic valve
2. Most commonly caused by *S. epidermidis*.

MORPHOLOGY

Q. Describe the morphology of infective endocarditis.

Q. Describe heart in infective endocarditis.

Gross (Figs 15.10 to 15.12):

Valves affected: Aortic and mitral valves (most common) and valves of the right heart (especially tricuspid) in intravenous drug abusers.

Vegetations: These are hallmark/pathognomonic of IE which have following features:

- **Appearance:** Friable, bulky, potentially destructive vegetations (less destructive in subacute than in acute endocarditis).

- **Number:** They may be single or multiple and may involve one or more than one valve.
- **Size:** Lesions vary in size from a small, superficial deposit to bulky, exuberant vegetations.
- **Changes in the involved valve:** Edema and inflammation and in severe cases, leaflet may perforate causing regurgitation.
- **Sites:** Vegetations form on:
 - Atrial side of the atrioventricular valves.
 - Ventricular side of the semilunar valves.
 - Points of closure of the leaflets or cusps.

IE: Vegetations are hallmark/pathognomonic.

Characteristic features of four major types of vegetative endocarditis are presented in Table 15.6.

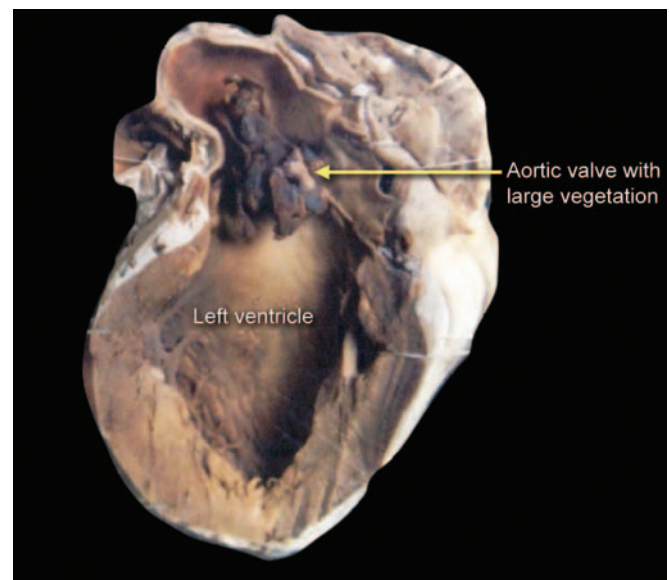


Fig. 15.10: Acute bacterial endocarditis. Aortic valve shows large friable vegetations

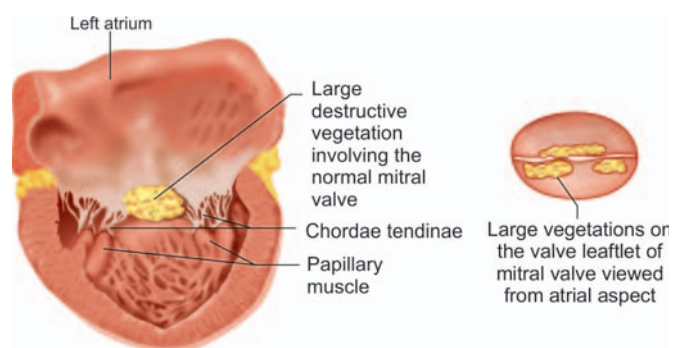


Fig. 15.11: Acute bacterial endocarditis. Mitral valve shows large friable vegetations

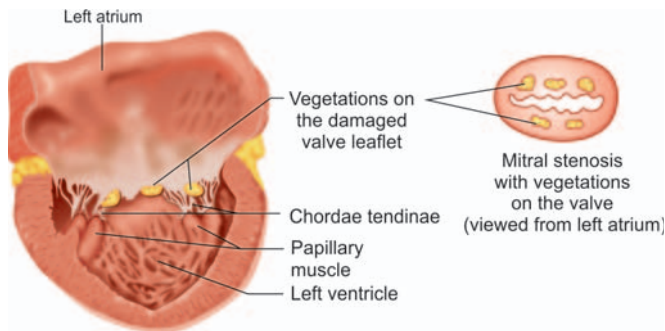


Fig. 15.12: Subacute bacterial endocarditis. Mitral valve shows vegetations

TABLE 15.6: Characteristic features of four major types of vegetative endocarditis

Type of endocarditis	Morphological feature
1. Rheumatic fever (RHD)	Small, warty vegetations along the line of closure of valve leaflets
2. Infective endocarditis (IE)	Large, irregular on the valve cusps that can extend on to the chordae tendinae
3. Nonbacterial thrombotic endocarditis (NBTE)	Small, bland vegetations usually attached to the line of closure of valve leaflets
4. Libman-Sacks endocarditis (SLE)	Small or medium-sized vegetations on either side of the valve leaflets

MICROSCOPY

- Vegetations are composed of platelets, fibrin, cell debris, inflammatory cells, and masses of (bacteria or other organisms).
- Vegetations of subacute IE may show granulation tissue at their bases indicative of healing. Later it may show fibrosis, calcification, and chronic inflammatory infiltrate.

Complications of Infective Endocarditis

Q. Describe the complications of infective endocarditis.

These may be divided into cardiac and extracardiac.

Complications of IE:

1. Cardiac
 - Embolic
 - Immune complex mechanism.
2. Extracardiac

Cardiac Complications

These are due to **direct valvular damage** and **consequences of local invasion**. The infection may spread locally from valve into the valve ring, adjacent mural endocardium or chordae tendineae.

1. **Ring abscess:** Vegetations may erode the **underlying myocardium** and produce an **abscess** which is known as ring abscess.
2. **Perforation and rupture:** May involve valve leaflets, aorta or interventricular septa (depending on the site of infection).
3. **Myocardial abscess.**
4. **Suppurative pericarditis.**
5. **Valvular dysfunction:** Stenosis or insufficiency.

Extracardiac Complications

Q. Extracardiac complications of subacute bacterial endocarditis.

Vegetations are usually friable and likely to be break, detach and cause embolism.

1. **Embolic complications (due to septic emboli):** Emboli contain large numbers of virulent organisms → **abscesses develop at the sites of arrest** of the emboli.
 - **Septic emboli from left side of the heart:** They enter systemic circulation and its consequences are:
 - **Septic infarcts (e.g. spleen, kidney or brain)**
 - **Mycotic aneurysms** (refer page 264)
 - **Small emboli (microthromboemboli) may produce tiny hemorrhagic lesions namely: (1) splinter or subungual hemorrhages and (2) Janeway lesions** which are small erythematous or hemorrhagic nontender, macular, lesions on the palms or soles.
 - **Septic emboli from right side of the heart:** They enter pulmonary circulation → lead to **pulmonary abscess**.
2. **Immunological phenomena**
 - **Focal segmental glomerulonephritis:** Develop due to **deposition of antigen-antibody complexes** in glomeruli. Grossly, the **outer surface of kidney** develops a **flea-bitten** appearance due to patchy hemorrhagic foci involving the glomeruli.
 - **Osler nodes:** They are small, **tender subcutaneous nodules in the pulp of the digits** and persist for hours to several days.
 - **Roth spots** are **retinal hemorrhages** with white or pale centers composed of fibrin.

Clinical Features

Infective endocarditis: Fever is the most consistent symptom.

- Symptoms:
 - **Fever** is the most common feature of IE.
 - Acute IE develops rapidly presenting with fever, chills, weakness, and lassitude.
- Clinical examination:
 - Murmurs may be heard either due to new valvular defect or a pre-existing cardiac disease.

Diagnostic Criteria for Infective Endocarditis (Box 15.2)

Infective endocarditis: Blood culture is positive for the organism in majority of the cases.

Duke criteria used for diagnosis of suspected infective endocarditis (IE). It requires either pathologic or clinical criteria.

Earlier diagnosis and effective treatment prevents complications.

BOX 15.2: Criteria for infective endocarditis

Pathologic Criteria

Demonstration of microorganisms by culture or histologic examination in

1. Vegetation
2. Embolus from a vegetation
3. Intracardiac abscess

Histological confirmation of active endocarditis in vegetation or intracardiac abscess

Clinical Criteria

Major

1. Blood culture(s) positive
2. Echocardiographic identification of a valve-related or implant-related mass or abscess
3. New valvular regurgitation

Minor

1. Fever
2. Predisposing heart lesion or intravenous drug use
3. Vascular lesions: Arterial petechiae, subungual/splinter hemorrhages, emboli, septic infarcts, mycotic aneurysm, intracranial hemorrhage, Janeway lesions
4. Immunological phenomena: Focal segmental glomerulonephritis, Osler nodes, Roth spots
5. Microbiologic evidence, including a single culture positive for an unusual organism

Clinical criteria required for diagnosis of IE: 2 major, or 1 major + 3 minor, or 5 minor criteria.

Infective endocarditis: The “gold standard” for the diagnosis of infective endocarditis is culture of organism from a valve or other endocardial surface.

Prognosis: It depends on the infecting organism and whether or not complications develop.

RHEUMATIC FEVER AND RHEUMATIC HEART DISEASE

Q. Define rheumatic fever.

Definition: Rheumatic fever (RF) is an **acute, post-streptococcal, immune-mediated, multisystem inflammatory disease**. It occurs a few weeks after an episode of group A streptococcal pharyngitis.

Two major phases:

1. **Acute rheumatic fever:** It frequently manifest as **acute rheumatic carditis**.

Old dictum is that “rheumatic fever licks the joint but bites the heart”.

2. **Chronic rheumatic heart disease:** Acute rheumatic carditis **may progress to chronic rheumatic heart disease (RHD)**. The deforming fibrotic valvular lesions (mainly mitral stenosis) are the principal/key features of chronic RHD.

Incidence

- **Age group:** Most common in **children between 5 to 15 years**.

RF: Most common affected age group is between 5 to 15 years.

- **Sex:** Both sexes are equally effected.
- **Socioeconomic conditions:** Incidence and mortality rate of RF and RHD have markedly decreased over the past century, due to improved socioeconomic conditions and rapid diagnosis and treatment of streptococcal pharyngitis.
- **Poor economy and overcrowding:** It is a predisposing factor in developing countries.
- Rheumatic fever rarely follows infections by streptococci at other sites, like skin.

Chronic Rheumatic Heart Disease

Q. Morphology of heart in chronic rheumatic heart disease (Sequale of rheumatic fever).

Rheumatic heart disease: Post-streptococcal disease in which antistreptococcal antibodies that cross-react with cardiac tissues.

Myocarditis and pericarditis usually resolve without any sequelae. But the endocarditis, mainly the **valvulitis induced damage is cumulative** and with each attack of rheumatic fever, it progresses → results in structural and functional alterations of the valves.

MORPHOLOGY

The lesions of rheumatic fever can be broadly divided into cardiac and extracardiac lesions.

A. Cardiac lesions:

Cardiac lesions can be studied under: (1) Acute rheumatic heart disease, and (2) Chronic rheumatic heart disease.

Q. Write short note on Aschoff bodies (Aschoff nodule).

Aschoff body (Fig. 15.13): It is **spheroidal or fusiform characteristic lesion** seen in rheumatic disease.

- **Size:** About to 1 to 2 mm.
- **Site:** During acute RF, may be found in any of the three layers, in the **perivascular interstitial region of the heart**.
- **Formation of Aschoff body consists of 3 phases:**
 - ◆ **Early/exudative phase:** It consists of a perivascular focus of swollen eosinophilic collagen surrounded by **lymphocytes, plasma cells, and macrophages**.
 - ◆ **Intermediate/granulomatous phase:** It is **pathognomonic of rheumatic myocarditis**. It is a **granulomatous lesion**, with a **central fibrinoid focus** associated with **lymphocytes** (primarily T-cells), **macrophages**, **Anitschkow cells** (pathognomonic for RF) and occasional plasma cells. **Anitschkow cells are plump activated macrophages**, which have abundant cytoplasm. They have round-to-ovoid nuclei in which the chromatin is disposed in a central, slender, wavy ribbon. These **nuclei resemble a caterpillar when cut longitudinally** (hence known as “**caterpillar cells**”). **On cross section**, these nuclei have an **owl-eye appearance**. Few Anitschkow cells may become **multinucleated** (with 2 to 4 nuclei) in which case, they are termed **Aschoff giant cells**.
 - ◆ **Late/healed /final phase:** Aschoff body is replaced by a nodule of **scar tissue**.

Acute rheumatic heart disease is characterized by **pancarditis**, involving all three layers of the heart (**endocardium, myocardium, and pericardium**).

1. **Endocarditis** (Fig. 15.14): Inflammation of the endocardium (valvular/mural) is known as endocarditis. It may involve valvular (valvular endocarditis) or mural endocardium (mural endocarditis).

RF: Verrucae are small, warty, firmly attached vegetations along the lines of closure of valve leaflets/tendinous cords.

- **Valvular endocarditis:**
 - ◆ During acute rheumatic carditis, **valve leaflets become inflamed and edematous**.
 - ◆ All four valves may be affected, but **left-sided valves (mitral and aortic) are more injured than do right-sided valves** (tricuspid and pulmonary).
 - ◆ Inflammatory damage causes focal loss of endothelium and **fibrin deposits along the lines of closure of the valve leaflets or along the tendinous cords**.
 - ◆ Overlying these deposits develop **small (1–2 mm) nodular or warty vegetations, called verrucae**. These verrucae are **firmly attached** and do not get dislodged. This is in contrast to friable detachable vegetations of infective endocarditis.

- **Mural endocarditis:** Inflammation of the mural endocardium is less prominent than valvular lesions and most common is **MacCallum plaque**.

Q. Write briefly on MacCallum plaque.

- ◆ **MacCallum plaques:** These are subendocardial lesions, which appear as **irregular, wrinkled, map-like thickenings** in the mural endocardium. Usually seen in the **posterior wall of the left atrium** (in chronic RHD and not in acute rheumatic fever), just **above the posterior leaflet of mitral valve**. These may be formed due to damage induced by chronic regurgitation of blood. **Microscopically**, they may show **multiple Aschoff bodies**.

MacCallum plaques: Irregular, wrinkled, map-like thickening seen in the posterior wall of left atrium in chronic rheumatic heart disease.

2. **Pericarditis:** Inflammation of the pericardium is known as pericarditis.
 - During acute RF, irregular deposits of fibrin are found on both visceral and parietal surfaces of the pericardium causing **fibrinous pericarditis**.
 - This fibrinous pericarditis, grossly resemble the shaggy surfaces of two slices of buttered bread that have been gently pulled apart. Hence, they are commonly called as **bread-and-butter pericarditis** (Fig. 15.15).
 - Clinically, It gives a friction rub, and it never causes constrictive pericarditis.
3. **Myocarditis:** Inflammation of the myocardium is known as myocarditis.
 - **Nonspecific myocarditis** during acute RF may cause dilation of heart. Microscopically, myocardium may show lymphocytes and macrophages or **Aschoff bodies**.
 - Few patients may die due to myocarditis.

Acute rheumatic fever: Mitral regurgitation during acute stage and mitral stenosis during chronic stage.

Acute rheumatic fever: The most common cause of death is due to myocarditis.

Acute rheumatic fever: Mitral valve is more commonly involved and responsible for 99% cases of acquired mitral stenosis.

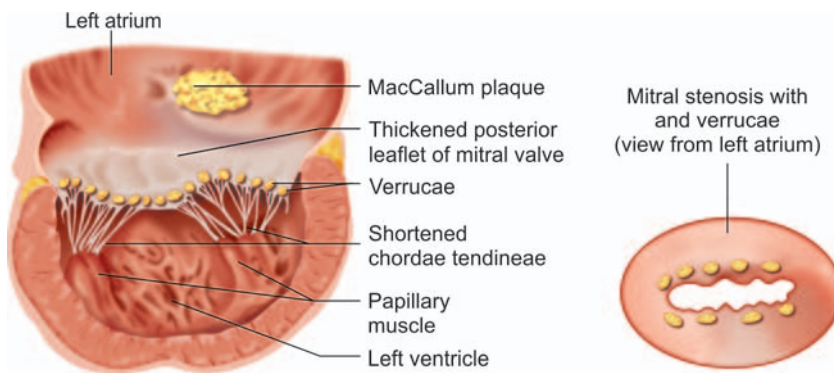
Valves Affected

- **Mitral valve** (Fig. 15.16) **alone:** It is the **most commonly (65–70% of cases) and severely affected valve** in chronic rheumatic disease.
- **Mitral valve along with the aortic valve** in another 25% of cases.
- **Tricuspid and pulmonary valve are rarely involved.**



Anitschkow cells:
Pathognomonic for RF.

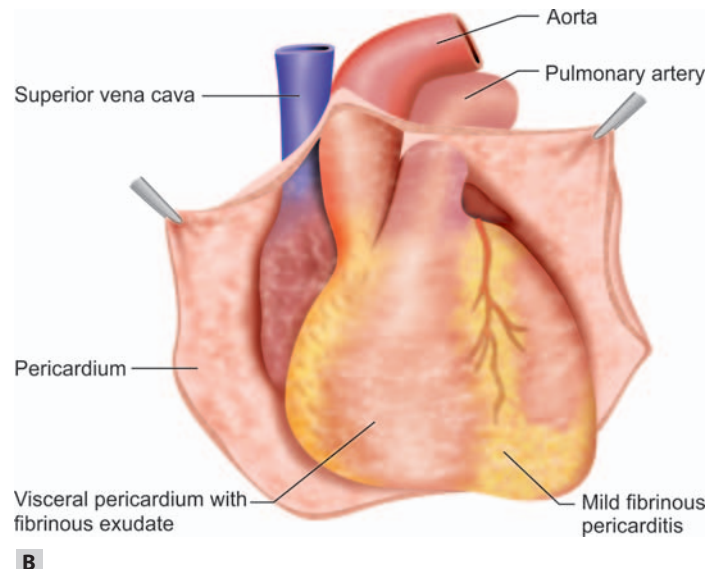
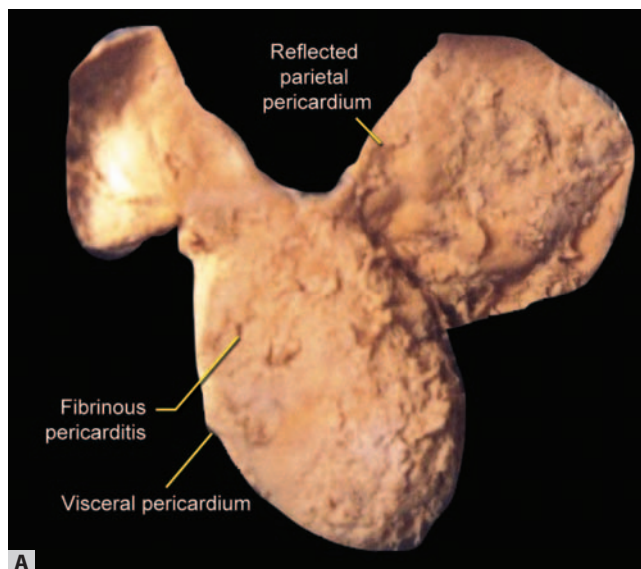
Figs 15.13A and B: (A) Aschoff body; (B) Magnified view of Anitschkow cells



Rheumatic fever: Pancarditis

1. Endocarditis
2. Pericarditis
3. Myocarditis.

Fig. 15.14: Gross appearance of mitral valve and left atrium in RHD



Figs 15.15A and B: Fibrinous pericarditis developing in rheumatic fever

Changes in the Affected Valves

1. Changes in mitral valve (Figs 15.16A and B):

Changes in the mitral valve:

Fusion of commissures

Fixed narrow opening (fish-mouth or button hole)

Shortening, thickening and fusion of the chordae tendineae.

- **Thickening of valve leaflet:** It is due to fibrosis following inflammation of the valve leaflets.
- **Fusion of commissures:** Healing of rheumatic lesions along the lines of closure leads to formation of fibrous adhesions/bridging of valvular commissures → result in a rigid and partially fused stenotic valve. In severe chronic rheumatic mitral valve disease, the valve orifice becomes reduced to a fixed narrow opening that has the characteristic appearance of a **fish mouth** (or **buttonhole**) when viewed from the ventricular aspect (Figs 15.16A and B).
- **Shortening, thickening and fusion of the chordae tendineae.**
- **Consequences of mitral stenosis** (Fig. 15.17):
 - ◆ **Left atrium progressively dilates** → favor mural thrombi in the appendage or along the wall.
 - ◆ **Thrombi can dislodge** → embolize.
 - ◆ **Chronic venous congestion in the lungs.**
 - ◆ **Pulmonary hypertension** → **right ventricular hypertrophy and right-sided heart failure.** Left ventricle is normal in isolated pure mitral stenosis.

2. Changes in aortic valve (Fig. 15.16B):

This is the **second most commonly involved valve** in rheumatic heart disease. Diffuse fibrous thickening of the cusps and fusion of the commissures cause **aortic stenosis**. The cusps may become calcified as the patient ages, resulting in **stenosis and insufficiency**.

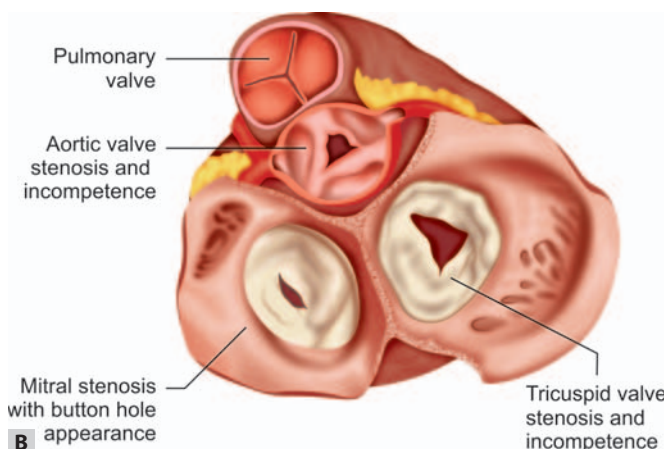
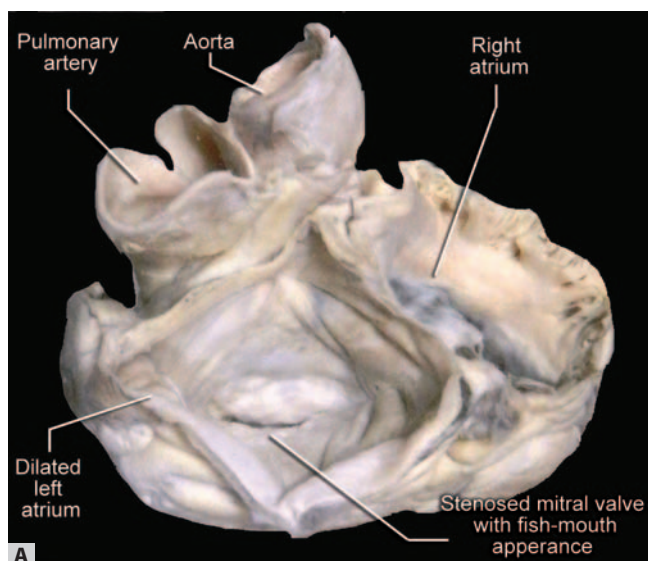
3. Tricuspid valve (Fig. 15.16B):

In cases of recurrent RF, the tricuspid valve may be deformed, always in association with mitral and aortic lesions.

4. Pulmonary valve: It is rarely affected.

Extracardiac Lesions (Fig. 15.17):

1. **Migratory polyarthritis:** It is the **usual presentation** in acute rheumatic fever and is accompanied by fever. In this, one large joint becomes painful and swollen, which then subsides and another joint gets involved for a period of days. They resolve spontaneously without any residual disability.
2. **Subcutaneous nodules:** These are usually seen in children than in adults. The nodules are oval to **spheroidal, painless** and measure **about 0.5 to 2 cm** in diameter. They are **attached to tendons or ligaments** and are usually located at the **extensor aspect of wrist, elbows, ankles or knees**. Microscopically, they appear like a giant Aschoff body.
3. **Erythema marginatum:** These skin lesions are **non-itchy reddish rashes** showing characteristic **bathing suit pattern of distribution**.
4. **Sydenham's chorea or Saint Vitus' dance:** It is a neurologic disorder with **involuntary rapid, jerky, purposeless movements of trunks and extremities**.
5. Rheumatic fever also may involve other organs like lung, pleura and arteries.



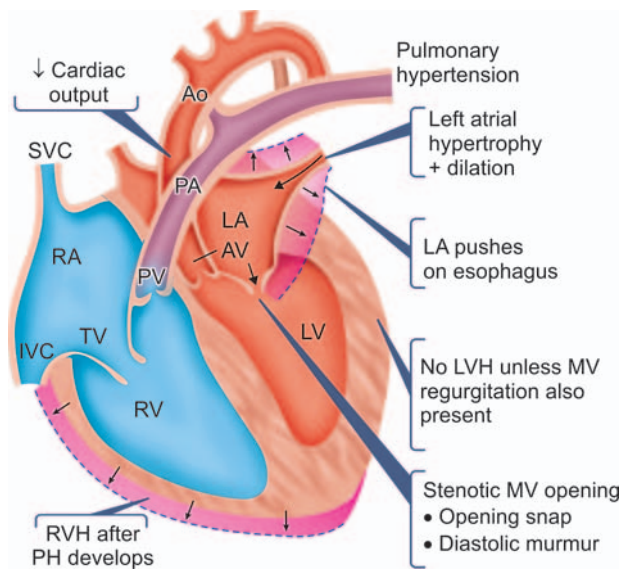
Figs 15.16A and B: Rheumatic valvular disease viewed from above. (A) Mitral stenosis with buttonhole deformity and dilated left atrium developed as a complication of chronic RHD; (B) Multivalvular rheumatic disease affecting mitral, tricuspid and aortic valve

Pathophysiology of mitral stenosis (Fig. 15.17):

- Narrowing of mitral valve → dilatation and hypertrophy of left atrium due to increased work in filling the ventricle during diastole.
- Chronic backup of atrial blood into the pulmonary vein → pulmonary venous hypertension → right-sided heart failure and right ventricular hypertrophy.
- Clinical features: Dyspnea and hemoptysis with rust-colored sputum (heart failure cells).

Mitral stenosis: Recurrent rheumatic fever is the most common cause.

Mitral stenosis → pulmonary hypertension → right-sided heart failure.



3Fs of mitral valve changes in rheumatic heart disease:

1. Fibrosis
2. Fixed narrow opening
3. Fusion of chordae tendineae.

Fig. 15.17: Consequences of mitral stenosis

Abbreviations: LA, left atrium; RA, right atrium; LV, left ventricle; RV, right ventricle; LVH, left ventricular hypertrophy; RVH, right ventricular hypertrophy; SVC, superior vena cava; IVC, inferior vena cava; TV, tricuspid valve; AV, atrioventricular valve; PV, pulmonary valve; PA, pulmonary artery

Extracardiac lesions of RF:

1. Migrating polyarthritis
2. Subcutaneous nodules
3. Erythema marginatum
4. Sydenham's chorea.

Etiology and Pathogenesis (Fig. 15.18)

Q. Describe the etiopathogenesis and pathology of rheumatic heart disease.

Acute **rheumatic fever** is a post-streptococcal disease. It develops after a latent period of 2 to 6 weeks after an episode of pharyngitis or tonsillitis by group A β -hemolytic streptococci. It occurs most often in children.

Immunologically mediated disease: Streptococcal infection introduces the streptococcal antigens into the body which activate both antibody and T-cell-mediated reactions against streptococci.

- **Antibody-mediated reactions (type II hypersensitivity reaction):** Antibodies may be produced by B-lymphocytes against various antigenic components of the *Streptococcus*. One of them produced against the **M proteins of streptococci** seems to **cross-react with certain similar self-antigens in the myocardial cells and glycoproteins of the valves in the heart**. This may be the mechanism for pancarditis in acute rheumatic fever.

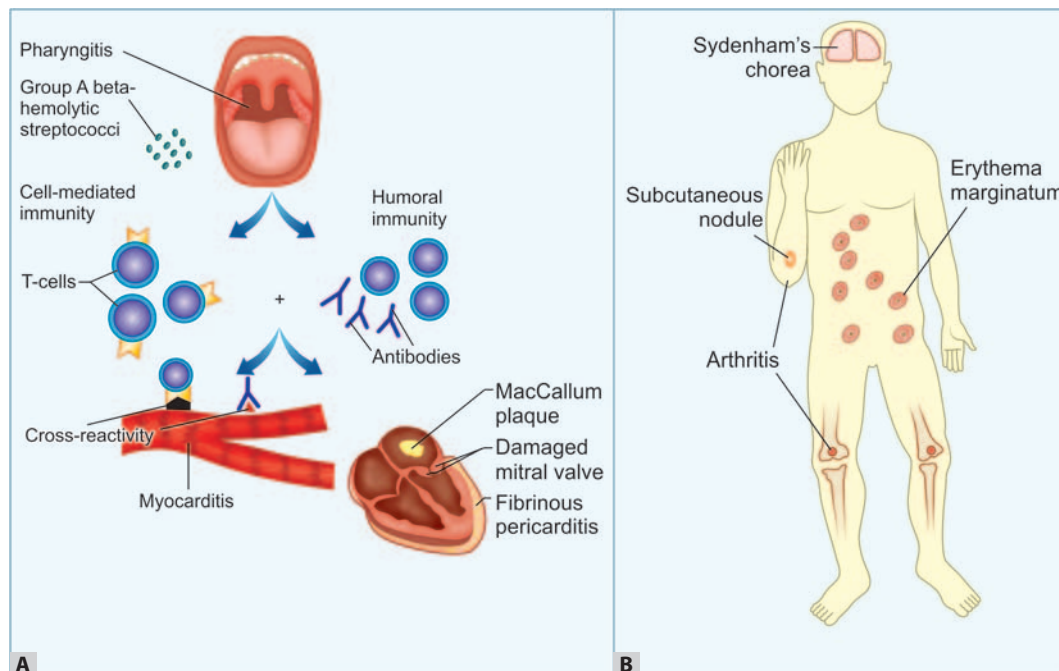


Fig. 15.18A and B: (A) Cardiac and (B) extracardiac lesions of rheumatic fever. Pharyngitis by β -hemolytic streptococci activates both humoral and cellular immunity. The antibodies produced against various antigenic components of the *Streptococcus*, can cross-react with cardiac antigens. The result is pancarditis (endocarditis, myocarditis, and pericarditis). Extracardiac lesions include polyarthritis, erythema marginatum, subcutaneous nodules and Sydenham's chorea

- **T-cell-mediated reactions (type IV hypersensitivity reaction):** CD4⁺ T-cells are also activated by streptococcal antigens. These along with antibodies **cross react with self-proteins in the heart**. These reactions produce cytokines leading to activation of macrophages, which are seen in lesions of rheumatic fever.

Acute rheumatic fever: Follows 3 weeks after the pharyngitis by β -hemolytic streptococci.

Acute rheumatic fever: Immune-mediated disease; type II and type IV hypersensitivity reaction.

Complications of Chronic Rheumatic Heart Disease

Q. Describe the complications of rheumatic heart disease.

- **Infective endocarditis:** The **damaged valves** of rheumatic heart disease provide a site for **deposition of bacteria**.
- **Mural thrombi:** They may be formed in **atrial or ventricular chambers**. Rarely, a large thrombus in the left atrial appendage may act as a ball valve and may obstructs the orifice of mitral valve.

Mitral stenosis: Most common cause is recurrent rheumatic fever.

- **Thromboemboli:** Mural thrombi may dislodge → form thromboemboli → **infarcts in various organs**.
- **Congestive heart failure:** It is associated with involvement of both mitral and aortic valves.
- **Adhesive pericarditis:** It follows the fibrinous pericarditis developed during acute attack.
- **Arrhythmias** (particularly atrial fibrillation): It is observed in mitral stenosis.

Atrial fibrillation: Common in mitral stenosis.

Clinical Features

Acute rheumatic fever: Most commonly presents with migrating polyarthritis.

Rheumatic fever usually manifests as a **migrating polyarthritis and/or carditis**. The **diagnosis is made by using Jones criteria** (Table 15.7): **Evidence of a preceding group A streptococcal infection**, with the presence of **two of the major criteria or one major and two minor criteria**.

Clinical findings of **acute carditis** include pericardial friction rubs, weak heart sounds, tachycardia, arrhythmias and various cardiac murmurs (type depending on the valve involved).

Acute rheumatic fever: Jones criteria used for the diagnosis.

TABLE 15.7: Jones criteria for diagnosis of rheumatic fever

Major criteria	Minor criteria
1. Pancarditis	1. Fever
2. Migratory polyarthritis of the large joints	2. Arthralgia
3. Sydenham's chorea	3. Elevated blood levels of acute-phase reactants, raised ESR and leukocytosis
4. Erythema marginatum	
5. Subcutaneous nodules	

Laboratory Findings

There is **no specific serological test** for rheumatic fever.

- **Serological tests:** **Pharyngeal cultures** for streptococci are **negative** by the time the illness begins. The antibodies to one or more streptococcal enzymes can be detected in the sera of most patients with RF. These include:
 - **Antistreptolysin O (ASO)**
 - **DNase B**
 - **Antihyaluronidase.**
- **Acute phase reactants**
 - **Raised ESR**
 - **C-reactive protein.**

Acute rheumatic fever: Raised ASO, DNase B titers, ESR and C-reactive protein.

Course

- **Recovery:** **Most acute attacks subside** with 4 to 6 weeks.
- **Recurrence:** Clinical manifestations reappear years or even decades after the initial episode of RF.

Prognosis: Long-term prognosis has improved the outlook due to surgical repair or prosthetic replacement of diseased valves.

CONGENITAL HEART DISEASE

Congenital means “born with”.

Congenital heart disease (CHD) is the **abnormalities of the heart or great vessels** that are **present from birth**.

Incidence

Congenital heart diseases are the **most important and common type of heart disease in children**.

Etiology and Pathogenesis

Most CHD arise from defective embryogenesis during 3 to 8 weeks of gestation, when major cardiovascular structures

form and begin to function. Two factors that are involved in CHD are:

1. **Genetic factors:** The main causes of congenital heart disease are sporadic genetic abnormalities. These may be:
 - **Single gene mutations**
 - **Small chromosomal deletions**
 - **Additions or deletions of whole chromosomes (trisomies and monosomies):** The most common genetic cause of congenital heart disease is **trisomy 21 (Down syndrome)**.

Most common genetic cause of congenital heart disease: Trisomy 21 (Down syndrome).

2. **Environmental factors:** These factors may be the primary cause congenital heart disease. **Examples:**
 - **Congenital rubella infection**
 - **Gestational diabetes**
 - **Exposure to teratogens** (including drugs)
 - **Nutritional factors:** During pregnancy, consumption of multivitamin containing folate may reduce the risk, whereas heavy consumption of alcohol increases the risk of congenital heart defects.
3. **Multifactorial:** Majority of CHD may be due to combination environmental and with genetic factors.

Clinical Features

- **Severe** congenital heart diseases result in **intrauterine death**.
- Some congenital heart diseases produce clinical manifestations soon after birth, but some may not be evident until adulthood (e.g. ASD).

Classification (Box 15.3)

Shunts: Abnormal communication between heart chambers or blood vessels. Shunts allow the flow of blood from high pressure to the low-pressure side.

The structural anomalies in congenital heart disease may be divided into four major categories: (1) right-to-left shunt, (2) left-to-right shunt, (3) an obstruction and (4) malposition of heart.

Left-to-right Shunt

These are characterized by the flow of blood from the left (systemic) side to the right (pulmonary) side of the circulation.

Consequences

- Left-to-right shunt causes **increased pulmonary blood flow** and initially does not produce cyanosis.

BOX 15.3: Classification of congenital heart diseases

Left -to-right shunt: Danger of shunt reversal if not corrected.

Type of congenital heart disease

1. **Left-to-right shunt**
 - Ventricular septal defect
 - Atrial septal defect
 - Patent ductus arteriosus
2. **Right-to-left shunt**
 - Tetralogy of Fallot
 - Transposition of the great arteries
 - Persistent truncus arteriosus
 - Tricuspid atresia
 - Total anomalous pulmonary venous connection
3. **Obstructive congenital heart disease**
 - Coarctation of the aorta
 - Aortic stenosis
 - Pulmonary stenosis
4. **Malposition of heart**—very rare
 - Dextrocardia

- But increased pulmonary blood flow **increases the volumes and pressures** in pulmonary circulation → results in **pulmonary hypertension** → causes **right ventricular hypertrophy**.
- Later, when the **pressure in right ventricle reaches systemic levels**, the shunt changes to a new **right-to-left**, which diverts the unoxygenated blood into the systemic circulation → results in cyanosis, and hence these CHD are known as **late cyanotic congenital heart disease, or Eisenmenger syndrome**.
- When the pulmonary hypertension becomes irreversible, the structural defects of congenital heart disease become irreparable.

Cyanosis: Blue discoloration of the mucous membranes resulting from an increased amount of reduced hemoglobin.

Right-to-left Shunt

If the pressure in the right side rises more than that in left, the blood flows from the right to left side (right-to-left shunt) → results in hypoxemia and **cyanosis** (blue discoloration of the skin and mucous membranes) due to the mixing of poorly oxygenated venous blood with systemic arterial blood (called **cyanotic congenital heart disease**).

Consequences

- **Paradoxical embolism:** It is a condition in which emboli from the peripheral veins can bypass the lungs and enter directly into the systemic circulation (refer page 98). It may result in infarction and abscess in the brain.

- **Clubbing and polycythemia:** Severe, long-standing cyanosis causes clubbing of the tips of the fingers and toes (called **hypertrophic osteoarthropathy**) and **polycythemia**.

Obstructive Congenital Heart Disease

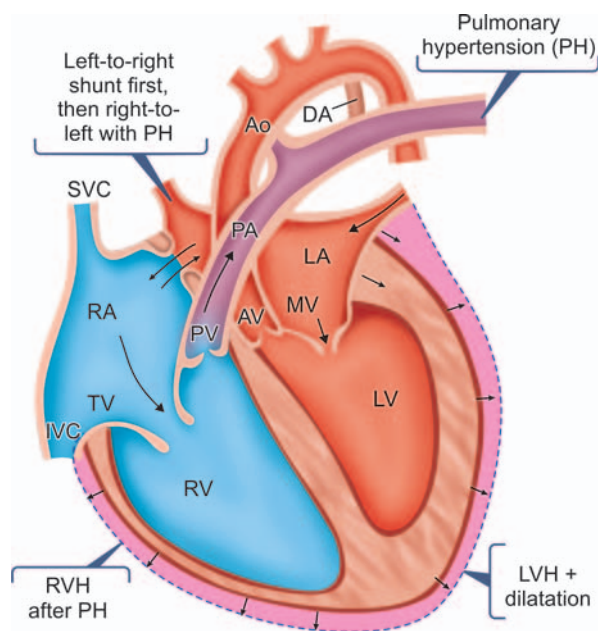
These are characterized by abnormal narrowing of chambers, valves, or blood vessels. **If the obstruction is complete, it is called an atresia.**

In some CHD, there may be coexistence of (e.g. Tetralogy of Fallot), both obstruction (pulmonary stenosis) and a shunt (right-to-left through a VSD).

Malposition of heart are very rare type of congenital heart diseases.

LEFT-TO-RIGHT SHUNTS

The common left-to-right shunt include ventricular septal defects (VSDs), patent ductus arteriosus (PDA), and atrioventricular septal defects (AVSDs).



ASD: Most common CHD in adults.

Fig. 15.19: Consequences of atrial septal defect

Abbreviations: LA, left atrium; RA, right atrium; LV, left ventricle; RV, right ventricle; LVH, left ventricular hypertrophy; RVH, right ventricular hypertrophy; SVC, superior vena cava; IVC, inferior vena cava; TV, tricuspid valve; AV, atrioventricular valve; PV, pulmonary valve; PA, pulmonary artery; Ao, Aorta; DA, ductus arteriosus

Atrial Septal Defect

An atrial septal defect (ASD) is an abnormal, fixed opening in the atrial septum. It is due to incomplete formation of tissue in the atrial septum, which allows flow of blood between the left and right atria. ASDs are **usually asymptomatic till adulthood**.

MORPHOLOGY

Classification: It is classified into three major types according to their location as: Secundum, primum, and sinus venosus.

- **Secundum ASDs (90%):** These result from a deficient or fenestrated oval fossa near the center of the atrial septum. The size varies, and may be single or multiple, or be fenestrated.
- **Primum anomalies (5%)** occur near the AV valves.
- **Sinus venosus defects (5%)** develop near the entrance of the superior vena cava.

Clinical Features

- ASDs cause a left-to-right shunt and results in increased pulmonary blood flow by about two to four times normal. Consequences of atrial septal defect are presented in Figure 15.19.
- A murmur may be heard due to increased blood flow through the pulmonary valve.
- ASDs usually do not produce symptoms before 30 years of age.

Complications: (1) heart failure, (2) paradoxical embolization, and (3) irreversible pulmonary vascular disease.

Prognosis: Mortality is low.

Ventricular Septal Defect

- Incomplete closure of the ventricular septum is known as ventricular septal defect (VSD), is the **most common form of congenital cardiac anomaly**.
- Most (70–80%) VSDs are associated with other congenital cardiac anomalies (e.g. tetralogy of Fallot).
- Cause free blood flow from the left-to-right ventricles.

Classification

Ventricular septal defect are classified according to their size and location.

- **Membranous VSD (90%):** They involve the membranous region of interventricular septum.
- **Infundibular VSD (10%):** They lie below the pulmonary valve or within the muscular part of the interventricular septum.

Clinical Features

The clinical features depend on the size of the defect.

- **Large ventricular septal defect**
 - Usually **membranous or infundibular type** and manifest from birth.
 - Cause **left-to-right shunt** → leading to right ventricular hypertrophy and pulmonary hypertension.
 - Irreversible pulmonary vascular disease develops later, in all patients resulting **reversal of shunt, cyanosis, and death**.
- **Small ventricular septal defect**
 - May not be recognized until adult life.
 - About 50% of small muscular VSDs may close spontaneously.

Consequences of ventricular septal defect are presented in Figure 15.20.

Patent Ductus Arteriosus

Normal Function of Ductus Arteriosus

In the fetus, it is an essential structure that carries the blood from the pulmonary artery to the aorta, thus bypasses the flow of blood to lungs (like the patent foramen ovale). After birth, it normally spontaneously closes and the blood from pulmonary artery flows to the lung.

Patent (Persistent) Ductus Arteriosus (PDA)

It is a congenital anomaly in which the **ductus arteriosus remains open after birth** (Fig. 15.21). PDAs may occur as an **isolated anomaly** (about 90%), or **associated with VSD, coarctation of the aorta, or pulmonary or aortic valve stenosis**. PDA produces a **characteristic continuous harsh murmur known as “machinery-like”**.

Clinical Features

Depends on diameter of PDA and the cardiovascular status of the patient. PDA is usually asymptomatic at birth, and a narrow PDA may not affect the child's growth and development. First, the shunt is from left-to-right and there is no cyanosis. But additional volume and pressure overload produces obstructive changes in small pulmonary arteries, leading to reversal of flow and its associated consequences.

PDA may be **either life threatening or lifesaving**.

Preservation of ductal patency (by administering prostaglandin E) is essential for survival of infants with congenital malformations that obstruct the pulmonary or systemic blood flow tracts. Example: In aortic valve atresia, preserved PDA supplies the major blood into the systemic circulation. Consequences of patent ductus arteriosus are presented in Figure 15.21.

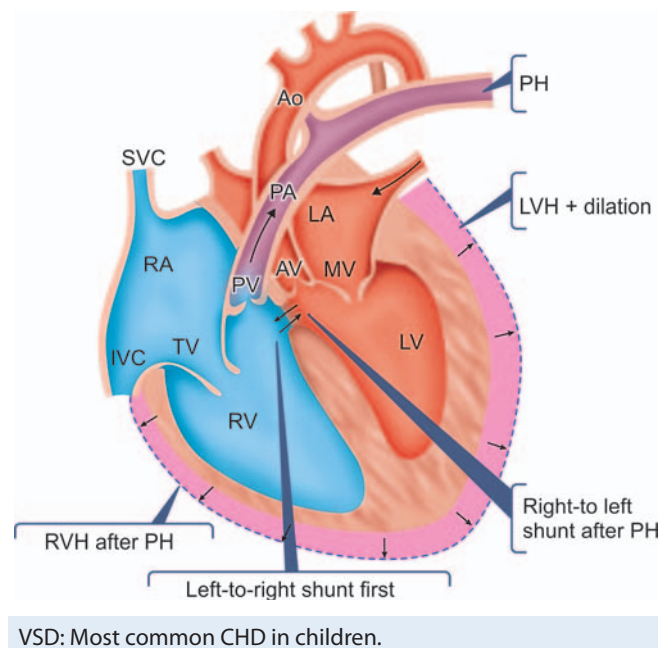


Fig. 15.20: Consequences of ventricular septal defect (refer Fig. 15.19 for meaning of abbreviations)

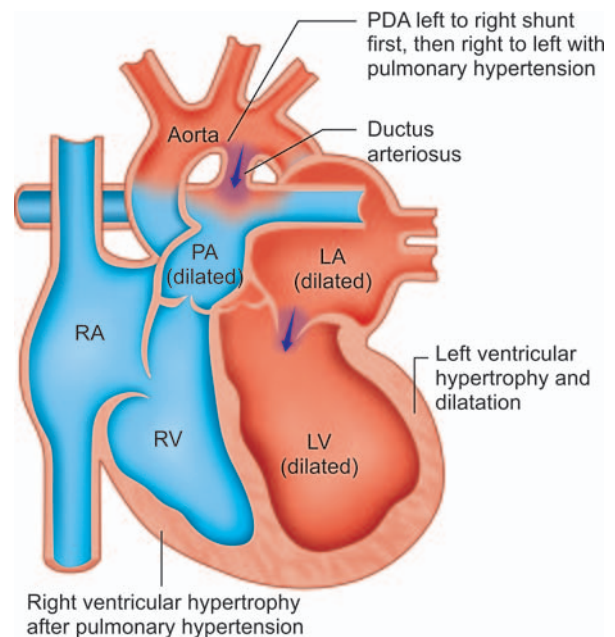


Fig. 15.21: Patent ductus arteriosus (PDA)

Patent Foramen Ovale

Normal Function of Foramen Ovale

- In the fetus, it is an essential small hole in the atrial septum at the oval fossa → produces a right-to-left shunt, though which the oxygen-rich blood from the placenta directly travels from the right to left atrium without passing through the non-inflated lungs.
- The **foramen ovale closes at birth** due to increased blood pressure on the left side of the heart.

Consequences of a Patent Foramen Ovale

Sustained pulmonary hypertension or transient increase in right-sided pressures (as occurs during a bowel movement, coughing, or sneezing), can produce transient right-to-left shunting, and paradoxical embolism.

RIGHT-TO-LEFT SHUNTS

These congenital diseases cause **cyanosis early in postnatal life** (cyanotic congenital heart disease). Tetralogy of Fallot, the most common, and others include transposition of the great arteries, persistent truncus arteriosus, tricuspid atresia, and total anomalous pulmonary venous connection.

Tetralogy of Fallot

Q. Write short note on tetralogy of Fallot.

Components (Fig. 15.22): The **four main features** of the tetralogy of Fallot (TOF) are:

1. **Ventricular septal defect:** Usually large.
2. **Subpulmonic stenosis/pulmonary valvular stenosis:** It causes obstruction of the right ventricular outflow tract.
3. **Aorta overriding the VSD** and both ventricular chambers.
4. **Right ventricular hypertrophy:** It is due to the obstruction to right ventricular outflow.

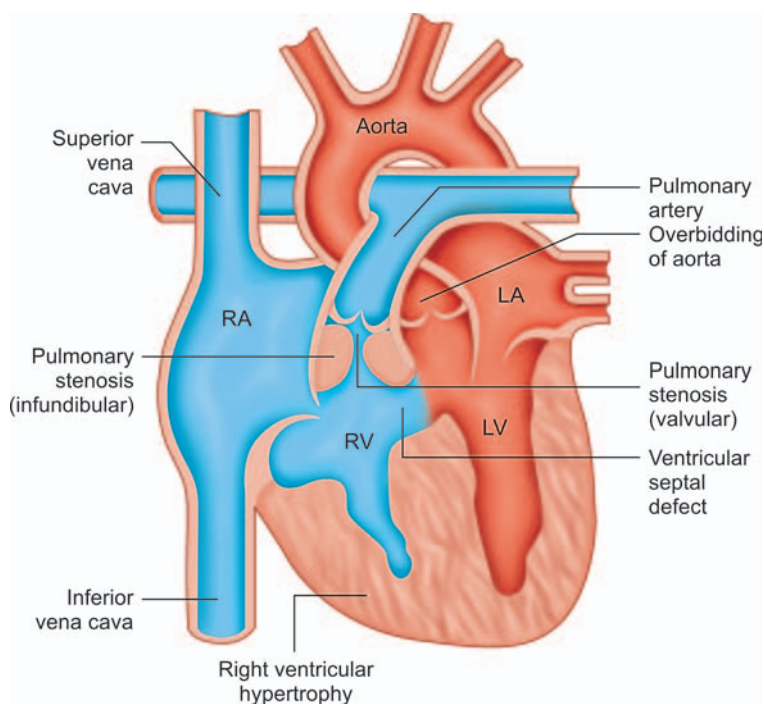
MORPHOLOGY

Heart is enlarged and may be **“boot-shaped”** due to marked right ventricular hypertrophy, particularly of the apical region.

Clinical Features

Clinical features depend mainly on the severity of the subpulmonary stenosis, because this decides the direction of blood flow.

- **Mild subpulmonary stenosis:** It clinically resembles an isolated VSD, and the shunt may be from left-to-right, **without cyanosis** (so-called pink tetralogy).



Tetralogy of Fallot: Most common cause of cyanotic congenital heart disease.

Tetralogy of Fallot: Causes cyanotic heart disease with boot-shaped enlarged heart.

Tetralogy of Fallot:

1. VSD
2. Subpulmonic stenosis aorta overriding the VSD
3. Right ventricular hypertrophy.

Fig. 15.22: Diagrammatic representation of tetralogy of Fallot. Four components are: (1) Ventricular septal defect; (2) Subpulmonic stenosis/pulmonary valvular stenosis; (3) Aorta overriding the VSD; (4) Right ventricular hypertrophy

- **Moderate subpulmonary stenosis:** It produces increased resistance to right ventricular outflow. When the right-sided pressures approach or exceed that of left-sided pressures → right-to-left shunt develops → **producing cyanosis** (classic TOF).
- **Severe subpulmonary stenosis:**
 - Pulmonary arteries become progressively hypoplastic with small and thin-walled.
 - Aorta becomes progressively larger in diameter.
 - As the child grows, the size of heart increases, but pulmonary orifice does not expand proportionally. This results in progressive worsening of the obstruction.
 - Majority of infants with TOF have cyanosis from birth or soon thereafter.

Transposition of the Great Arteries (TGA)

Aorta arises from the right ventricle and lies anterior and to the right of the **pulmonary artery**, which **originate from the left ventricle**.

- The heart chambers and its relations are normal (concordant), i.e. the right atrium joins the right ventricle and the left atrium empties into the left ventricle.

MORPHOLOGY (FIG. 15.23)

- **Right ventricle** shows **hypertrophy**, because it functions as the systemic ventricle.
- **Left ventricle** shows **atrophy** (thinning of its wall) because it supports only the low-resistance pulmonary circulation.

Outlook: It depends on three factors.

- Degree of mixing of the blood
- Severity of the tissue hypoxia
- Ability of the right ventricle to maintain the systemic circulation.
 - Without surgery, most patients die during infancy.

Transposition of great vessels:

1. Aorta arises from right ventricle
2. Pulmonary artery arises from left ventricle
3. Atria normal.

Transposition of great arteries:

Survival is incompatible unless accompanied by another minor congenital defect.

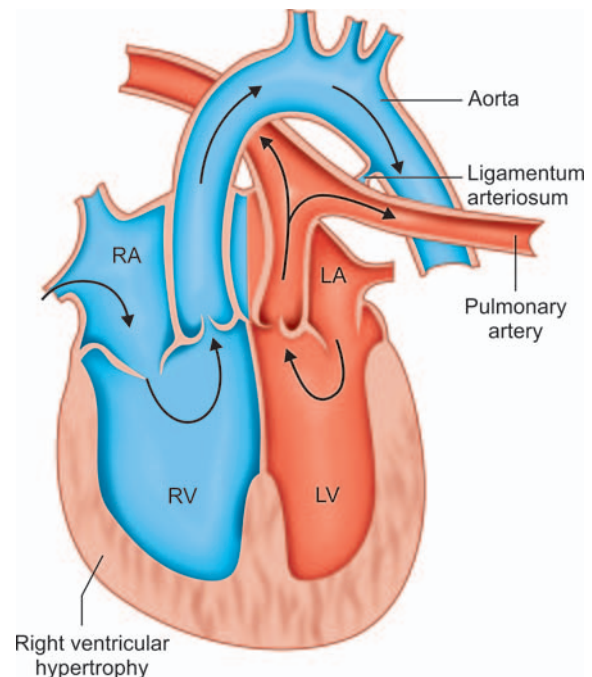


Fig. 15.23: Transposition of great arteries

OBSTRUCTIVE CONGENITAL ANOMALIES

Congenital obstruction to blood flow may occur at:

- **Heart**
 - Heart valves: For example, stenosis or atresia of the aortic or pulmonary valves.
 - Chamber of heart: For example, subpulmonary stenosis in TOF.
- **Great vessel:** For example, coarctation of the aorta.

Coarctation of the Aorta (Aortic Coarctation)

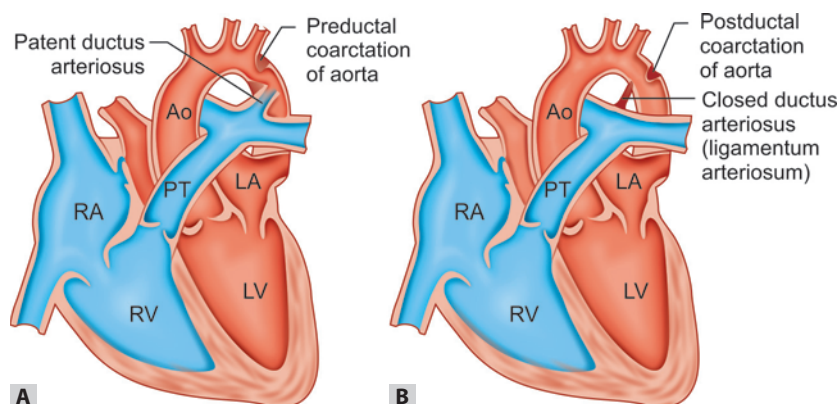
Coarctation = Narrowing or constriction.

Coarctation (**narrowing, constriction**) of the aorta is the common structural anomalies.

Sex: It is common in male than in females (2:1). But females with Turner syndrome may have a coarctation.

Coarctation of aorta: May be:

1. Isolated anomaly or
2. Associated with other anomalies.



Figs 15.24A and B: Coarctation of aorta. (A) With patent ductus arteriosus; (B) Without patent ductus arteriosus

Collateral in coarctation: Develops between intercostal arteries above and below the constriction.

Types

Coarctation of aorta:

1. Infantile/preductal
2. Adult/postductal.

Two classic forms.

1. **Infantile (preductal) form** (Fig. 15.24A): It is characterized by tubular hypoplasia of the aortic arch proximal to a patent ductus arteriosus. It produces symptoms in early childhood.
2. **Adult (postductal) form** (Fig. 15.24B): It shows narrowing of the aorta, opposite the closed ductus arteriosus (ligamentum arteriosum) distal to the arch vessels.

Extent of narrowing of the aortic lumen varies from minimal narrowing to maximal narrowing with only small channel. Coarctation of the aorta may occur as a solitary defect, or accompanied by other defects such as bicuspid aortic valve, congenital aortic stenosis, ASD, VSD, mitral regurgitation, or berry aneurysms of the circle of Willis in the brain.

Infantile coarctation: Associated with Turner syndrome.

Clinical Features

- Depend on the severity of the narrowing and the patency of the ductus arteriosus.
- **Coarctation of the aorta with a patent ductus arteriosus** (Fig. 15.24A)
 - It usually manifests immediately after birth.
 - Through patent ductus arteriosus, unsaturated (deoxygenated) blood from pulmonary artery is delivered to the aorta distal to coarctation. This produces **cyanosis only in the lower half of the body**. The upper half of the body is supplied by great vessels, which originate proximal to coarctation.

Adult coarctation: Blood pressure difference between upper and lower extremity is > 10 mm Hg

- **Coarctation of the aorta without a patent ductus arteriosus** (Fig. 15.24B):
 - **Most are asymptomatic**, and may not be recognized till adult life.
 - It presents with **hypertension in the upper extremities**; and **weak pulses and hypotension in the lower extremities**.
 - The lower extremities may show features of arterial insufficiency (i.e. claudication and coldness).
 - In adults, **collateral circulation may develop between the pre-coarctation arterial branches and the post-coarctation arteries**.
 - These appear as enlarged intercostal and internal mammary arteries, which may produce **radiographically visible erosions (“notching”) of the undersurfaces of the ribs**.

Collateral in coarctation: Develops between intercostal arteries above and below the constriction.

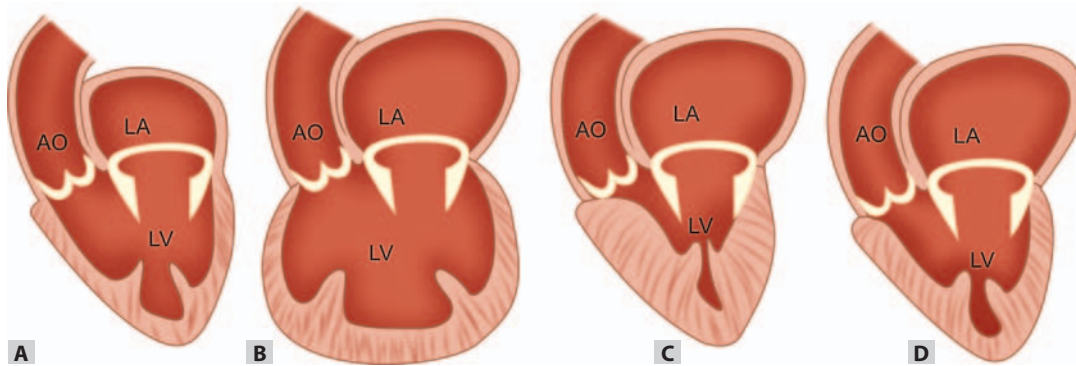
- Coarctations may be associated with **murmurs throughout systole**; sometimes with a thrill. The heart is enlarged (cardiomegaly) due to left ventricular pressure-overload hypertrophy.

CARDIOMYOPATHY

Q. Define and classify cardiomyopathy.

Definition

- Cardiomyopathies are a **heterogeneous group of diseases of the myocardium that affect the mechanical or electrical function of the heart**.



Figs 15.25A to D: Three major morphologic patterns of cardiomyopathy: (A) Normal (B) Dilated cardiomyopathy; (C) Hypertrophic cardiomyopathy (D) Restrictive cardiomyopathy

Abbreviations: AO, Aorta; LA, Left atrium; LV, Left ventricle

- Cardiomyopathy term should be restricted to the conditions which primarily affect the myocardium. It does not include myocardial involvement due to congenital, acquired valvular, hypertensive, and coronary arterial or pericardial abnormalities.

Etiology

They can be genetic/inherited or have infective, toxic causes or idiopathic.

Classification

Cardiomyopathies may be classified according to a variety of criteria, including the underlying genetic basis of dysfunction. Two fundamental forms of cardiomyopathy are:

1. **Primary cardiomyopathy:** Consists of heart muscle disease predominantly involving the myocardium and/or of unknown cause. Three major forms include dilated, hypertrophic and restrictive type of cardiomyopathy (Figs 15.25A to D).
2. **Secondary cardiomyopathy:** Consists of myocardial disease of unknown cause or associated with systemic disease (e.g. chronic alcohol use, amyloidosis).

CARDIAC MYXOMA

Q. Write short note on cardiac myxoma.

- Myxomas are the **most common primary cardiac tumor** of the adult heart.
- They are **benign tumors** arising from primitive multipotent mesenchymal cells.
- **Site:** About 90% of myxomas arise in the atria, **more common in the left atrium** in the region of the fossa ovalis in the atrial septum.
- **Number:** **Usually single**, but can rarely be multiple.
- **Size:** Range from **small (<1 cm)** to **large (≥10 cm)**
- **Appearance:** **Sessile or pedunculated**. Vary from globular hard masses with hemorrhage to soft, translucent, papillary, or villous lesions having a gelatinous appearance. The pedunculated type may be mobile during systole into the atrioventricular valve producing intermittent obstruction.
- **Microscopy:** Composed of stellate or globular myxoma cells embedded within an abundant acid mucopolysaccharides ground substance.
- **Clinical features:** Mainly due to **valvular “ball-valve” obstruction, embolization**, or constitutional symptoms, such as fever and malaise. Treatment is by surgical removal.

Spider cell: Cardiac rhabdomyoma.

Lung Disorders

OBSTRUCTIVE LUNG DISEASES

Obstructive lung diseases consists of four diseases namely (i) emphysema, (ii) chronic bronchitis, (iii) asthma and (iv) bronchiectasis. Chronic obstructive pulmonary/lung disease (COPD) is defined as a disease state characterized by airflow limitation that is not fully reversible. COPD includes two main diseases namely: **Emphysema** and **chronic bronchitis**.

Emphysema

Emphysema: Chronic obstructive airway disease characterized by permanent dilatation of air spaces distal to terminal bronchioles.

Q. Define emphysema.

Definition: Emphysema is a **chronic lung disease** characterized by abnormal **irreversible** (permanent) **dilatation of the airspaces distal to the terminal bronchiole**. This is associated with destruction of their walls but without obvious fibrosis. Recent evidences show that small airway fibrosis is present which contributes to airflow obstruction.

Types of Emphysema/Classification (Fig. 16.1)

Emphysema: Major subtypes are: (i) centriacinar, (ii) panacinar, (iii) paraseptal and (iv) irregular.

Q. Classify/mention the types of emphysema.

Emphysema is **classified according to its anatomic distribution** (location of the lesions) within the lobule into **four major types**: (i) centriacinar, (ii) panacinar, (iii) paraseptal and (iv) irregular.

1. Centriacinar (centrilobular) emphysema

- Dilatation involve the **central or proximal parts of the acini** (formed by respiratory bronchioles), whereas

distal alveoli are spared. Thus, both emphysematous and normal airspaces are present within the same acinus and lobule.

- **Site:** Common and severe in the **upper lobes**, especially in the **apical segments**.
- **Association:**
 - Occurs in **heavy smokers** and in association with **chronic bronchitis** and **coal-worker's pneumoconiosis**.
 - Walls of the emphysema often show **black pigment**.
 - **Inflammation around bronchi and bronchioles** is common.

Centriacinar emphysema:

1. Involves proximal parts of acini (spares alveoli)
2. Severe in upper lobes
3. Smokers
4. Seen as most common type clinically.

2. Panacinar (panlobular) emphysema

- **All the air spaces beyond terminal bronchiole** are more or less uniformly/equally dilated.
- **Site:** In contrast to centriacinar emphysema, more common in the **lower lobes**, and is usually **most severe at the bases**.
- **Association:** With α_1 -antitrypsin (α_1 -AT) deficiency.

Panacinar emphysema:

1. Involves all the air spaces beyond terminal bronchiole (entire acinus)
2. More common and severe in lower lobes
3. Associated with α_1 -antitrypsin deficiency.

Panacinar emphysema: Prefix "pan" refers to the entire acinus but not to the entire lung.

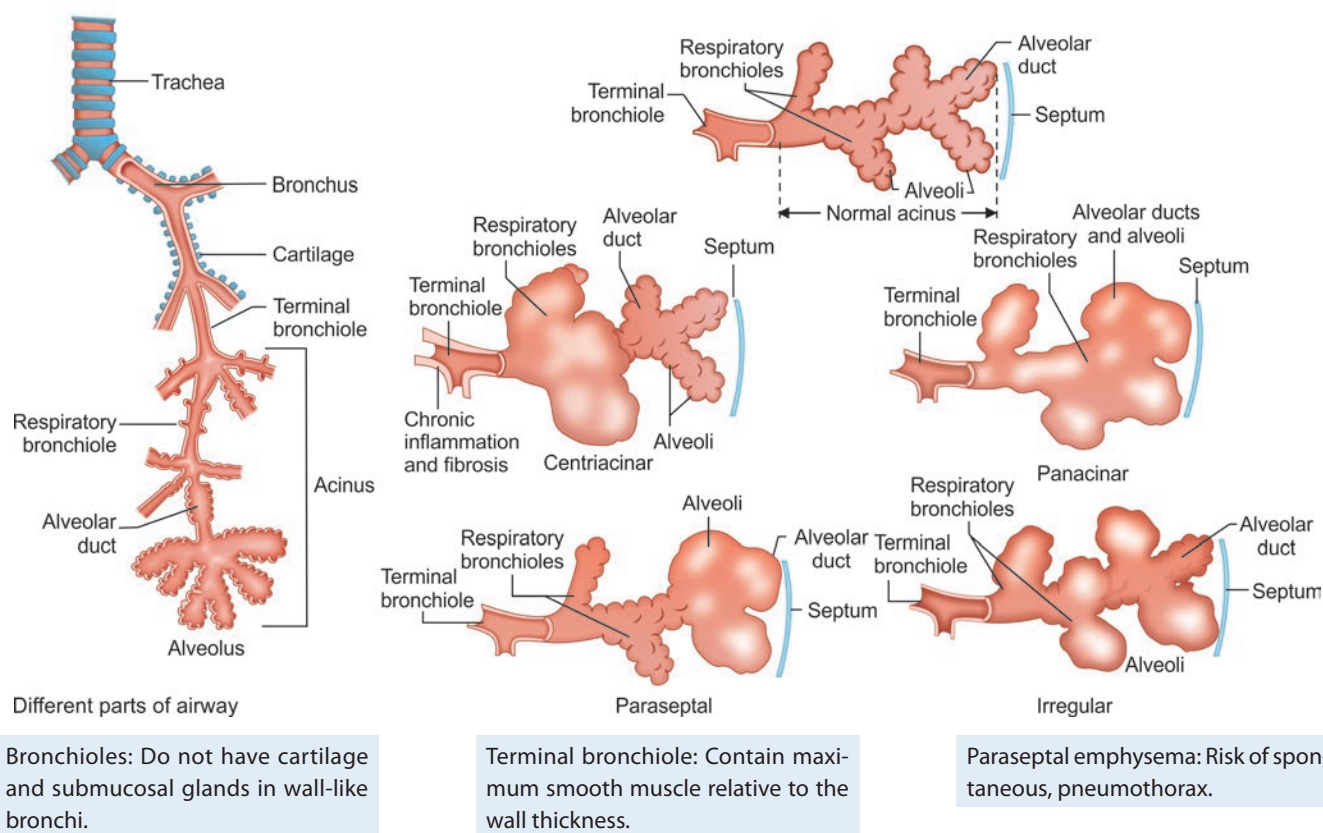


Fig. 16.1: Four major types of emphysema

3. Distal acinar (paraseptal) emphysema

- Dilatation affects the **distal air space** at the periphery of the lobule and the proximal portion is normal.
- **Dimension:** It forms multiple, enlarged airspaces ranging from **less than 0.5 cm to more than 2.0 cm in diameter**. Dilated spaces of more than 1 cm in size are known as **bullae** which may rupture and cause spontaneous pneumothorax.
- **Site:** More common **adjacent to septa, pleura** and at the **margins of the lobules**. **More severe in the upper lobes**.
- **Association:** It results from forces pulling on the septa and occurs **adjacent to areas of fibrosis, scarring, or atelectasis**.

Distal acinar (paraseptal) emphysema: Involves distal air spaces and are prone to spontaneous rupture → pneumothorax.

4. Irregular (scar or cicatricial) emphysema

- Acinus is **irregularly involved** and may be asymptomatic.
- **Most common form** of emphysema.
- **Association:** Occurs **near the scar** and is commonly found **around old healed inflammatory process like tuberculous scars**.

Irregular (scar or cicatricial) emphysema:

1. Acinus is irregularly involved
2. Associated with scars/fibrosis
3. Histologically most common type.

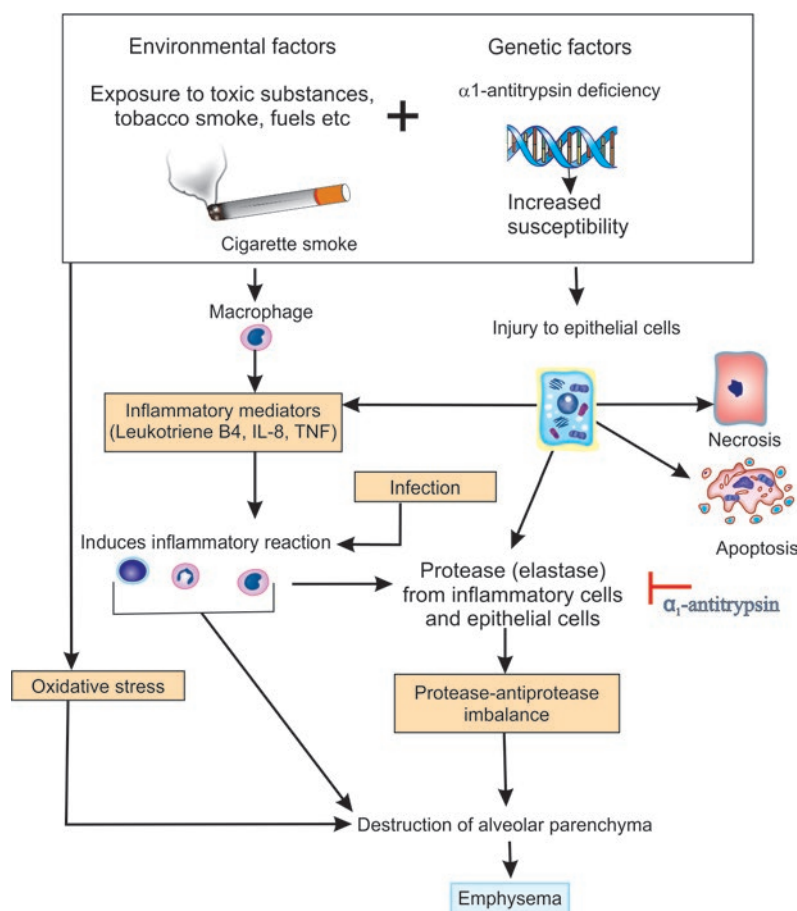
Etiology and Pathogenesis (Fig. 16.2)

Q. Etiopathogenesis of emphysema.

Clear-cut association between heavy cigarette smoking and development of emphysema is observed. Inhaled cigarette smoke and other toxic substances produce damage to the lung and produces inflammation. This leads to destruction of the lung parenchymal (emphysema) and disease of the airway (bronchiolitis and chronic bronchitis). The major event in emphysema is destruction of alveolar wall. Factors involved in the pathogenesis of emphysema are as follows:

1. Inflammatory mediators and leukocytes

- **Inflammatory mediators:** Many inflammatory mediators are released in the lung (e.g. leukotriene B₄, IL-8, TNF). The sources of these mediators are resident epithelial cells and macrophages.
- **Attraction of leukocytes:** Chemotactic factors (e.g. leukotriene) attract inflammatory cells from the circulation and exaggerate the inflammatory process



Emphysema: Most important etiologic agent is smoking.

Pathogenesis of emphysema: Inhaled environmental toxins (e.g. cigarette smoke) induce inflammation → releases elastases, oxidants and cytokines → destroy alveolar wall.

Emphysema: Imbalance between

- Protease and antiprotease
- Oxidants and antioxidants.

Fig. 16.2: Pathogenesis of emphysema. Exposure to environmental toxins (e.g. cigarette smoke) causes inflammatory reaction, cell death and proteolysis of extracellular matrix (ECM). α_1 -antitrypsin (α_1 -AT) deficiency also result in increased degradation of ECM

Abbreviations: IL-8, interleukin 8; TNF, tumor necrosis factor

(proinflammatory cytokines) and produce structural changes (due to growth factors).

2. **Protease-antiprotease imbalance:** Injury (e.g. by smoking or other toxic substances) activates and favor accumulation of inflammatory cells (e.g. neutrophils) and damages the epithelial cells. This leads to local release of proteases by inflammatory cells and damaged epithelial cells. The proteases can damage the connective tissue. Normally, the damage by proteases is prevented by antiprotease (e.g. α_1 -antitrypsin is a major inhibitor of proteases). About 1% of patients with emphysema have deficiency of protective antiproteases.

α_1 -antitrypsin deficiency:

- Autosomal recessive disorder
- Tendency to develop emphysema.

- **Genetic deficiency of α_1 -antitrypsin:** α_1 -antitrypsin is normally present in serum, tissue fluids and macrophages. A balance is maintained between

protease and antiproteases. α_1 -antitrypsin deficiency is inherited as autosomal recessive disorder that exhibits polymorphism. In these patients with absence of α_1 -antitrypsin activity, there is excessive digestion of elastic tissue which produce emphysema. The damage is aggravated by smoking.

3. **Oxidative stress:** Contents of tobacco smoke, damage to the alveoli (apoptosis) and inflammatory cells produce oxidants. They cause further damage to the tissues and aggravate inflammation.
4. **Infection:** Though not involved in initiation of emphysema bacterial and/or viral infections, may exacerbate the existing inflammatory process and chronic bronchitis.

Small airways obstruction in emphysema:

- Due to loss of elastic tissue in the walls of alveoli that surround respiratory bronchioles.
- Small airway inflammation may narrow the bronchiolar lumen.

MORPHOLOGY

Emphysema: Grossly, large voluminous lung. Microscopically, shows large alveoli separated by thin septa.

Gross (Fig. 16.3)

- Usually, the **upper two-thirds of the lungs** are more severely involved.
- **Bullae** are found in irregular and distal acinar emphysema.
- Advanced emphysema produces **voluminous lungs**.
- Emphysema can easily be seen on the cut surface of formalin-inflated fixed lung.

Microscopy (Fig. 16.4)

- Lung shows **abnormally large alveoli** separated by thin septa. There is destruction of alveolar walls and loss of attachments of the alveoli to the outer wall of small airways. The pores of Kohn are enlarged.
- In advanced disease, **bullae may be seen**.
- There may be inflammatory changes in small airways.

Clinical Course

Emphysema: Patients characteristically have adequate oxygenation of hemoglobin and are called as pink puffers.

Manifestations appear late until at least one-third of the functioning pulmonary parenchyma is damaged.

- **Dyspnea** begins insidiously and steadily progresses.
- **Cough and expectoration.**
- **Weight loss** is common.

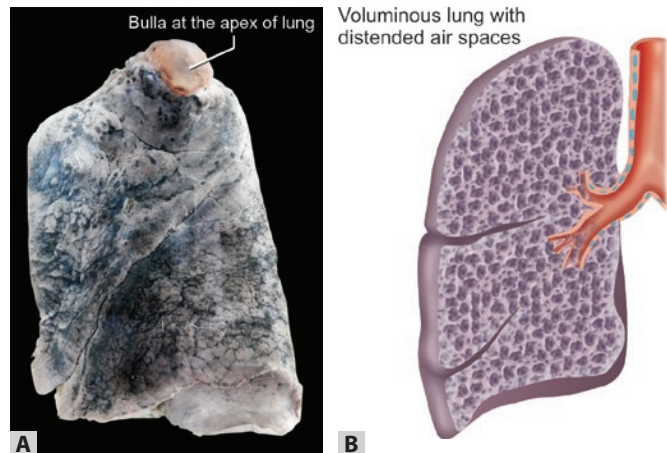
Clinical examination: Barrel-shaped chest, dyspnea with prolonged expiration, and breathes through pursed lips. Expiratory airflow limitation is helpful in diagnosis.

Complications: (i) **Cor pulmonale**, (ii) **congestive heart failure** due to secondary pulmonary hypertension and pneumothorax.

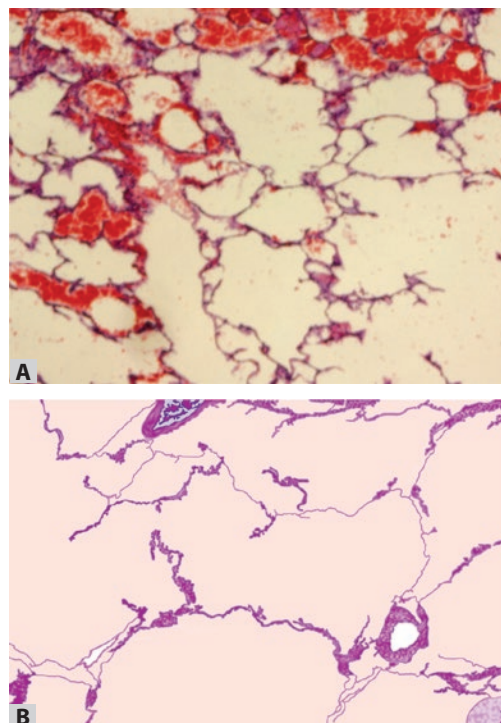
Cause of death: (i) Respiratory acidosis and coma, (ii) right-sided heart failure and (iii) massive collapse of the lungs secondary to pneumothorax.

Other Forms of Emphysema

- **Compensatory hyperinflation:** It is characterized by **dilation of alveoli without destruction of septal walls**. It develops as a compensatory response to loss of lung substance elsewhere (e.g. removal of a diseased lung or lobe).
- **Bullous emphysema (Fig. 16.3A):** The air spaces more than 1 cm in diameter in the distended state are known as blebs or bullae. They can occur in any type of emphysema. They **may rupture and produce pneumothorax**.



Figs 16.3A and B: Voluminous lung in emphysema; (A) Grossly voluminous lung with bulla on the apex of lung; (B) Cut section (diagrammatic)



Figs 16.4A and B: Microscopy of emphysema with destruction of alveolar walls forming large alveoli. (A) H & E; (B) Diagrammatic

Bulla: Distended air spaces more than 1 cm in diameter

- **Interstitial emphysema:** It is characterized by the **entry of air into the connective tissue stroma** of the lung, mediastinum, or subcutaneous tissue. It may result from alveolar tears in pulmonary emphysema, which occurs during coughing.

CHRONIC BRONCHITIS

Definition: Chronic bronchitis is defined clinically as **persistent cough with sputum** production (chronic productive cough) **for at least 3 months in at least 2 consecutive years**, in the absence of any other identifiable cause.

Chronic bronchitis: Persistent productive cough for at least 3 months in at least two consecutive years, in the absence of any other identifiable cause.

Etiology

Chronic bronchitis: Cigarette smoking is the most important etiological risk factor.

- **Cigarette smoking:** It is the most important risk factor.
- **Air pollutants:** Sulfur dioxide, nitrogen dioxide.
- **Toxic industrial inhalants:** Occupational dust exposure.
- **Respiratory tract infection:** It may initiate and promote chronic bronchitis.

Pathogenesis

Q. Discuss the pathogenesis of chronic bronchitis.

- **Irritation by inhaled air pollutants:** The initiating factor is irritation of large airways mucosa by inhaled air pollutants, such as **tobacco smoke** (90% of patients are smokers), **dust, cotton, sulfur dioxide, nitrogen dioxide and silica**. Irritants cause inflammation → infiltration by CD8+ T lymphocytes, macrophages and neutrophils.
- **Hypersecretion of mucus:**
 - **Hyperplasia/hypertrophy of the submucosal glands in large airways (trachea and bronchi):** Develops in response to inhaled **environmental irritants and proteases released from neutrophils** (e.g. elastase, cathepsin histamine and IL-13). This leads to **hypersecretion of mucus**.
 - **Marked increase of goblet cells in small airways (small bronchi and bronchioles):** They produce **excessive mucus** → mucus plugging of bronchial lumen → inflammation and fibrosis of bronchial wall → **leads to airway obstruction**.

Both the submucosal gland hypertrophy and the increase in goblet cells are thought to be protective metaplastic response against air pollutants (e.g. tobacco smoke, sulfur dioxide and nitrogen dioxide).

Role of Inflammation

- Inhalants producing chronic bronchitis cause damage to cells and cause acute as well as chronic inflammation

(neutrophils, lymphocytes and macrophages). Chronic inflammation and accompanying fibrosis involving small airways can produce chronic airway obstruction.

Role of Infection

- Cigarette smoke predisposes to infection in several ways.
- **Infection may be secondary** rather than primary and probably important in maintaining and producing acute exacerbations.
- **Infection may cause direct damage to airway epithelium** and interferes with ciliary action of the respiratory epithelium → **defective clearance of bacteria** by leukocytes.

MORPHOLOGY

Gross

Airways (large and small) changes include:

- **Mucous membrane:** It shows **hyperemia, swelling and edema**. It appears dusky red.
- **Lumen:** They are filled with excessive **mucus or mucopurulent** secretion.

Chronic bronchitis: Microscopically shows

1. Hyperplasia of submucosal glands
2. Increase in goblet cells
3. Squamous metaplasia
4. Fibrosis of bronchiolar walls

Microscopy (Fig. 16.5):

Major characteristic features are as follows:

- **Hyperplasia and hypertrophy of mucus-secreting glands in the submucosa:** This results in **shift of type of gland from normal mixed seromucous to pure mucous** in the trachea and bronchi → results in **hypersecretion of mucus**.
- **Reid index:** It is the **ratio of the thickness of the mucous gland layer to the thickness of the wall** between the base of the surface epithelium and the inner limit of the cartilage plates. It is useful for detecting the increase in the size and number of the mucus glands. Reid index (normally 0.4) is increased in chronic bronchitis.
- **Chronic inflammation of the airways** with predominant infiltration by lymphocytes.
- **Surface epithelium:** May show **mild increase in the numbers of goblet cells** and focus of **squamous metaplasia**.
- **Marked narrowing of bronchioles:** It is due to
 - **Excess mucus in the airways.**
 - **Thickening of the bronchial wall** due to hyperplasia of mucus glands, edema, inflammation, and fibrosis.
 - In severe cases, there may be obliteration of lumen due to fibrosis (**bronchiolitis obliterans**).

Chronic bronchitis: Reid index (normal 0.4) is increased.

Reid index: Ratio of mucus gland layer thickness to the thickness of wall between epithelium and cartilage.

Clinical Features

Usually affects **middle-aged men** who are **heavy smokers**.

- **Early symptoms:** **Persistent productive cough** (sputum) for many years.
- **Later stage:**
 - **Dyspnea on exertion**
 - **Blue bloaters:** Patients develop **hypercapnia, hypoxemia, and mild cyanosis**. Such patients are called 'blue bloaters.'
 - **Pink puffers:** These patients have more emphysema than bronchial obstruction. Therefore, they **hyperventilate to produce** a relatively **normal blood gas profile**.

Chronic bronchitis:

- Blue bloater
- Retain carbon dioxide
- Develop cyanosis.

Emphysema:

- Pink puffer
- Blow off carbon dioxide.

Complications

- Progression to **chronic obstructive pulmonary disease (COPD)**.
- Longstanding cases lead to **cor pulmonale** with cardiac failure.
- May develop squamous **metaplasia and dysplasia** of the respiratory epithelium → a rich soil for cancerous transformation.

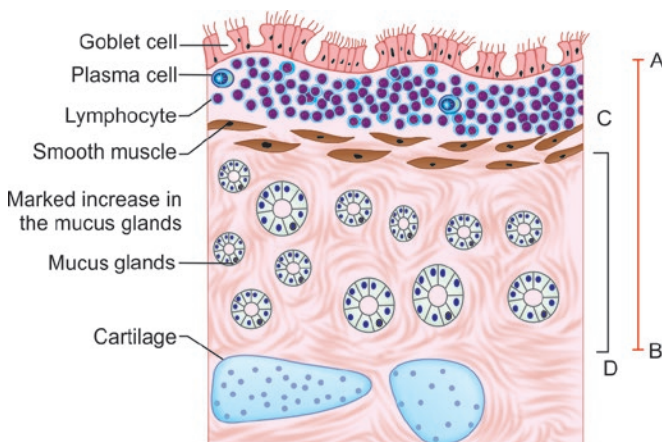


Fig. 16.5: Microscopic features of chronic bronchitis. The bronchial submucosa is markedly expanded by hyperplasia of submucosal glands and constitutes more than 50% of the thickness of the bronchial wall. The Reid index equals the maximum thickness of the bronchial mucus glands internal to the cartilage (C to D) divided by the bronchial wall thickness (A to B)

ASTHMA

Asthma: Disorder of the bronchial tree characterized by reversible bronchoconstriction in response to various stimuli.

Definition: Asthma is a **chronic inflammatory disorder** of the bronchial tree (airways) in which **breathing is periodically rendered difficult** by **widespread narrowing of the bronchi** (reversible bronchoconstriction). It is **clinically characterized by recurrent episodes of wheezing, breathlessness, tightness of the chest and cough**.

Asthma is common, but is relatively rare cause of death. It usually develops at night and/or in the early morning.

Characteristics

1. **Episodes of bronchoconstriction**—Partly reversible, either spontaneously or after treatment.
2. **Inflammation of the bronchial walls.**
3. **Increased mucus secretion.**

Classification (Box 16.1)

Q. Classify bronchial asthma.

BOX 16.1: Classification of asthma

According to type of antigen

1. **Atopic** (allergic/extrinsic)
2. **Non-atopic/intrinsic** (without evidence of allergen sensitization)

According to the agents or events that trigger bronchoconstriction

1. Seasonal
2. Exercise-induced
3. Drug-induced (e.g. aspirin)
4. Occupational asthma
5. Asthmatic bronchitis in smokers

Common Types of Asthma

Atopic Asthma

- **Most common type** of asthma that usually begins in **childhood**.
- **Family history of asthma or allergic diseases** (e.g. eczema, urticaria, or hay fever) is common.
- Classic example of **type I IgE-mediated hypersensitivity reaction**.
- Exogenous substances (**allergens**) causing asthma **can be recognized**.

- **Triggering environmental allergens:** It includes **dusts, pollens, animal dander and foods.**
- **Skin test** with the causative allergen **results in an immediate wheal-and-flare reaction.**

Non-atopic Asthma

- **No causative exogenous factors** can be identified and it does not show allergen sensitization.
- **Skin tests** are usually **negative.**
- **Family history** of asthma is **less common.**
- **Triggering events:**
 - **Respiratory infections due to viruses** (e.g. rhinovirus, parainfluenza virus).
 - **Inhaled air pollutants** (e.g. smoke, fumes).
- It may be **due to hyper-irritability of the bronchial tree.**

Drug-induced Asthma

Many drugs may trigger asthma (e.g. aspirin-sensitive asthma). Aspirin inhibits cyclooxygenase pathway of arachidonic acid metabolism → produces leukotrienes (bronchoconstrictor) → causes asthma.

Occupational Asthma

Triggering occupational agents:

- **Fumes** (epoxy resins, plastics).
- **Organic and chemical dusts** (wood, cotton, platinum).
- **Gases** (toluene).
- **Other chemicals** (formaldehyde, penicillin products).

Etiology

Q. Discuss the pathogenesis of asthma.

Endogenous Risk Factors

- **Genetic predisposition:** Major etiological factor in atopic asthma is genetic predisposition to **type I hypersensitivity (atopy) reaction** and exposure to environmental trigger. One of the **susceptibility locus is on the chromosome 5 (5q)** → several genes involved in regulation of IgE synthesis and mast cell and eosinophil growth and differentiation.
- **Airway hyper-responsiveness:** It is an abnormality in which there is **excessive tendency for airways to contract** (bronchoconstrictor) too easily in response to multiple inhaled triggers that usually does not have any effect on normal individuals.
- **Gender:** More common in boys than girls and, after puberty, women slightly more commonly than men.
- **Age:** Most cases **begin before the age of 25 years.**

Environmental Risk Factors: Asthma Triggers

Several stimuli trigger asthmatic attacks:

- **Inhaled allergens:** These include house dust, mites and house hold pets. They may cause allergic sensitization and asthma in early childhood.
- **Upper respiratory tract viral infections:** e.g. rhinovirus, respiratory syncytial virus and coronavirus.
- **Air pollution:** e.g. sulfur dioxide, ozone and diesel particulates. Indoor pollutants, such as exposure to nitrogen oxides from cooking stove.
- **Passive smoking:** It may trigger asthma.
- **Drugs:** e.g. β -adrenergic blockers and aspirin.

Hygiene Hypothesis

It proposes that individuals with **lack of infections in early childhood** are more prone to asthma than children brought up on farms who are exposed to a high level of endotoxin. Intestinal parasite infection may also be associated with a decreased risk of asthma.

Pathogenesis of Atopic Asthma (Fig. 16.6)

Q. Discuss the pathogenesis of atopic bronchial asthma.

Major Etiological Factors in Atopic Asthma

- **Genetic predisposition to type I hypersensitivity (atopy).**
- **Acute and chronic airway inflammation.**
- **Hyperresponse to various environmental antigens (allergens).**

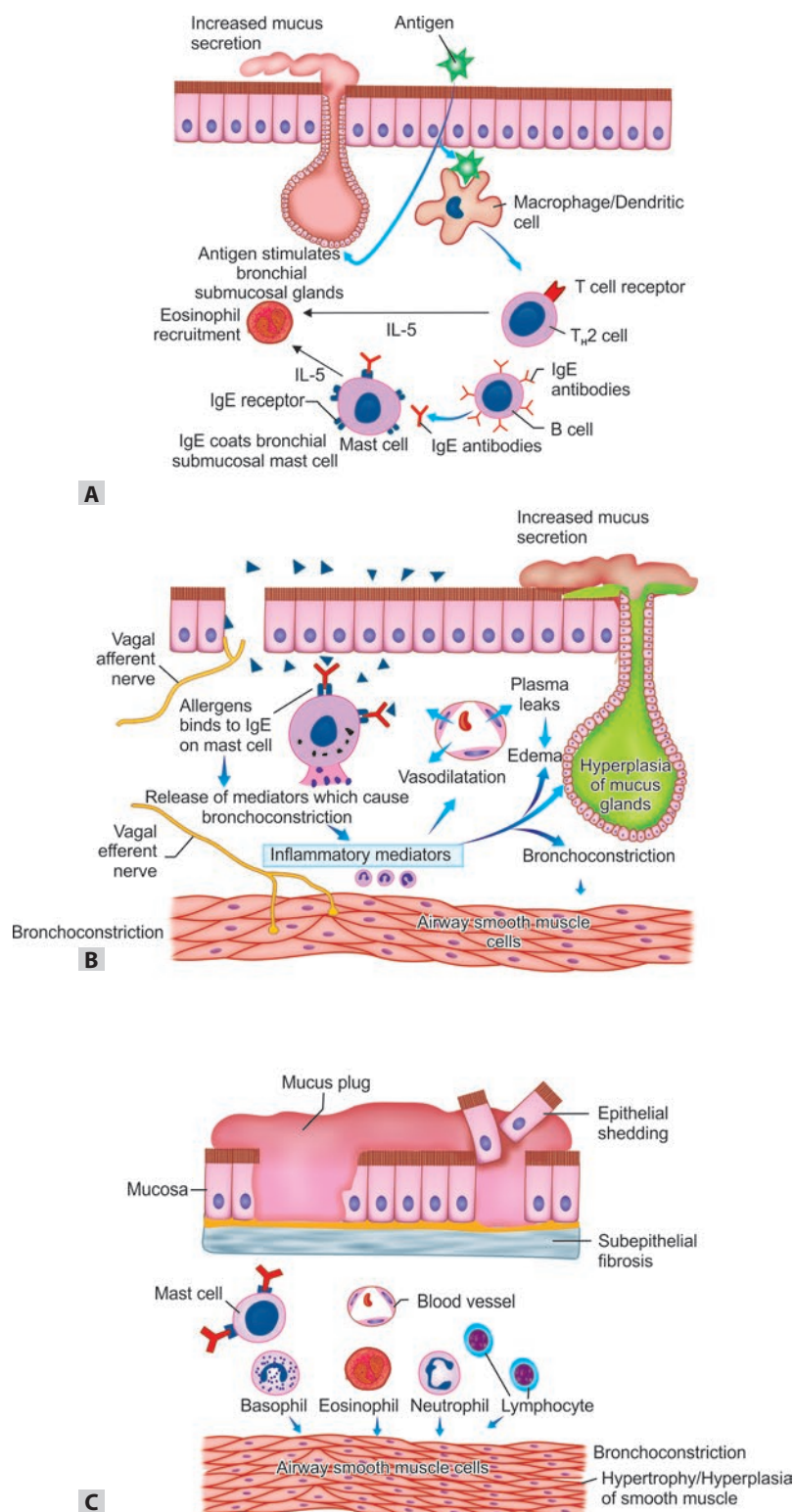
Inflammation involves many cell types and inflammatory mediators. **Genetic predisposition** with susceptibility genes makes individuals prone to develop **strong T_H2 reactions against environmental antigens** (allergens).

- **T_H2 cells secrete cytokines:** These cytokines promote allergic inflammation and **stimulate B cells to produce IgE** and other antibodies. The cytokines include:
 1. **IL-4:** Stimulates the production of IgE.
 2. **IL-5:** Activates eosinophils.
 3. **IL-13:** Stimulates mucus secretion from bronchial submucosal glands and **promotes IgE production by B cells.**

Steps in Pathogenesis

Sensitization

- **Genetically predisposed** individuals are sensitized against many **environmental antigens** (allergens).
- The allergen is taken by antigen presenting cells (macrophage/dendritic cell) and **elicits a T_H2 -dominated response.** This promotes **production of IgE by B cells.**



Figs 16.6A to C: Pathogenesis of asthma. (A) Sensitization: Inhaled allergens (antigen) are taken up by antigen presenting cells (macrophage/dendritic cell) and elicit a T_H2 -dominated response favoring IgE production and eosinophil recruitment; (B) On re-exposure to antigen (allergen), they bind to IgE bound on mast cells and release preformed mediators from mast cells. These mediators, either directly or via vagal stimulation produce bronchospasm, increased vascular permeability, and mucus production; (C) The recruitment of leukocytes (neutrophils, eosinophils, and basophils; lymphocytes and monocytes) signals the beginning of the late phase and a fresh round of mediator released from leukocytes, endothelium, and epithelial cells. Major basic protein, eosinophil cationic protein also causes damage to the epithelium

Extrinsic asthma:

- Family history
- Type I hypersensitivity reaction.

Intrinsic asthma:

- No family history
- Normal serum IgE levels
- Non-immune.

Atopic asthma: Caused by T_H2 and IgE-mediated type I hypersensitivity reaction to environmental antigens known as allergens.

Atopic asthma: T_H2 cells secrete cytokines IL-4, IL-5 and IL-13 that are important mediators.

IL-13 gene polymorphism:

Strongly associated with bronchial asthma.

Atopic asthma: Two main steps namely sensitization and reactions (early and late).

Asthma: Eosinophils are the most important inflammatory cells in most of the types of asthma.

Asthma: Airway damage caused by major basic protein secreted by eosinophils.

- IgE produced during sensitization **coats bronchial submucosal mast cells**. IL-5 secreted by T_H2 cells and mast cells **recruit eosinophils**. Allergen stimulates bronchial submucosal glands to secrete mucus.

On re-exposure

- **The allergens binds to IgE bound to the bronchial submucosal mast cells** → activates mast cells → immediately **release bronchoconstrictor mediators from the mast cell granules**.
- Mast cells releases **preformed mediators and produce cytokines** → responsible for the early-phase (immediate hypersensitivity) reaction and the late-phase reaction.
 - **Early reaction:** It is characterized by **bronchoconstriction, increased mucus production, and vasodilation with increased vascular permeability** (causes edema). These are produced either directly by the **mediators** released from mast cells **or through stimulation of vagal receptors** in the subepithelium.
 - **Late-phase reaction:** It is characterized by:
 - ◆ Inflammation: Recruitment of leukocytes (eosinophils, neutrophils, basophils, lymphocytes and monocytes) takes place by fresh mediators released by mast cells, epithelial cells and leukocytes. Example, (i) eotaxin—produced by airway epithelial cells, is a potent chemoattractant and activator of eosinophils, (ii) chemokines promote recruitment of more T_H2 cells, and (iii) major basic protein of eosinophils causes damage to epithelial cells and more constriction of airway.
 - ◆ Airway remodeling (refer under microscopy).

Refer type I hypersensitivity reactions on page 118.

MORPHOLOGY

Gross

- **Airways** (bronchi and bronchioles) are **occluded by plugs** of thick, tenacious mucus.
- In patients who die due to status asthmaticus, the lungs are greatly distended due to overinflation, and shows small areas of atelectasis (collapse).

Microscopy

Major processes which contribute to the narrowing of airways are as follows:

1. **Increased amount of mucus forms mucus plugs** and are seen in the bronchioles or bronchi. **Mucus plug contains desquamated epithelial cells and eosinophils and forms spiral-shaped cast of the airways** and are called **Curschmann spirals**. These may be found in the sputum.
2. **Inflammation:** **Numerous eosinophils** are found in the walls of the the bronchi and bronchioles. **Charcot–Leyden crystals**, which are **crystalloid derived from an eosinophil lysophospholipase binding protein called galectin-10** are also found. There is also edema and congestion of the airways.

3. **Airway remodeling:** It is the **group of structural changes in the bronchial wall due to repeated bouts of inflammation** and consists of:

- An **increase in size and number** (hypertrophy/hyperplasia) of the **submucosal glands**.
- **Hypertrophy and/or hyperplasia of the bronchial wall smooth muscle**.
- **Increased vascularity**.
- **Deposition of subepithelial collagen** accompanied by fibrosis and thickening of the basement membrane.

Clinical Course

- Acute asthmatic attack usually lasts **up to several hours**.
- In some **mild degree of chest tightness, dyspnea, wheezing and cough** with or without sputum production, may constantly present.
- **Between the asthmatic attacks**, patients may be **asymptomatic**.
- **Status asthmaticus:** It is the **most severe form of asthma** in which the severe acute paroxysm persists for days and even weeks. The **bronchoconstriction does not respond to the drugs**. It may cause **severe airflow obstruction leading to severe cyanosis and even death**.

Diagnosis

- Demonstration of an increase in airflow obstruction (from baseline levels).
- Difficulty with exhalation (prolonged expiration, wheeze).
- **Elevated eosinophil count** in the peripheral blood.

Q. Add a note on sputum findings in bronchial asthma.

- **Sputum:** It is viscous and yellow and is **rich in eosinophils**. Microscopy shows:
 - **Charcot–Leyden crystals** are mainly seen in atopic asthma. They have the shape of a pair of **long, narrow, six-sided pyramids placed base-to-base**.
 - **Curschmann spirals** are **corkscrew-shaped twist of condensed mucus several centimeters long** that is usually **surrounded by an elongated mass of clear or opalescent material**.
 - **Creola bodies** are **compact clusters or strips of columnar epithelial cells** shed from the bronchus.

Three Cs of sputum findings in asthma:

1. Curschmann spirals
2. Charcot–Leyden crystals
3. Creola bodies.

Charcot–Leyden crystals: Crystalloid derived from an eosinophil lysophospholipase binding protein called galectin-10.

Charcot–Leyden crystals: Crystalloid derived from an eosinophil lysophospholipase binding protein called galectin-10.

Curschmann spirals: Mucus plug containing desquamated epithelial cells and eosinophils and forms spiral-shaped cast of the airways.

BRONCHIECTASIS

Q. Define bronchiectasis.

Definition: Bronchiectasis is a disorder characterized by **irreversible** (permanent), **abnormal dilation of bronchi and bronchioles**. Bronchiectasis may be either a focal (localized) or a diffuse (generalized) process.

Bronchiectasis: Characterized by irreversible, abnormal dilation of bronchi and bronchioles.

Etiology

Q. Discuss the etiopathogenesis of bronchiectasis.

The dilatation of bronchi and bronchioles is caused by **destruction of the muscle and elastic tissue of bronchial wall**. It represents a secondary disorder as the end stage of many unrelated disorders. It may be divided into obstructive and nonobstructive (postinflammatory).

Nonobstructive (Postinflammatory) Bronchiectasis

It is **usually of diffuse type** characterized by dilatation throughout the lung.

Postinfectious

It may be the result or associated with **chronic necrotizing infections**.

- **Bacteria** (*Mycobacterium tuberculosis*, *Staphylococcus aureus*, *Haemophilus influenzae*, *Pseudomonas*)
- **Viruses** (adenovirus, influenza virus, human immunodeficiency virus [HIV]).
- **Fungi** (*Aspergillus* species).

Focal nonobstructive bronchiectasis usually develops as a **complication of childhood infections, such as measles and pertussis**.

Reversible bronchial dilation may be seen in viral and bacterial pneumonia.

Genetic Causes

Q. Kartagener's syndrome.

1. **Kartagener or immotile cilia syndrome (primary ciliary dyskinesia):** It is an **autosomal recessive** syndrome with **lack of ciliary function** (due to absence of inner or outer dynein arms of cilia) and causes retention of secretions and recurrent infections. It comprises of **sinusitis, bronchiectasis, dextrocardia** (with or without situs inversus) and **male infertility** (sperm dysmotility).

Kartagener or immotile cilia syndrome:

- Autosomal recessive
- Lack of ciliary function
- Sinusitis, bronchiectasis, dextrocardia (with or without situs inversus) and male infertility (sperm dysmotility).

2. **Young's syndrome:** Patients develop bronchiectasis, sinusitis and obstructive azoospermia. The cause is not known.
3. **Other dyskinetic ciliary syndromes:** These have deficient cilia throughout the body and include radial spoke deficiency ("Sturges syndrome") and absence of the central doublet of cilia.

Immunodeficiency

- Patients with hypogammaglobulinemia, HIV infection, bronchiolitis obliterans after lung transplantation have increased susceptibility to infections → localized or diffuse bronchiectasis.

Autoimmune and Immune-mediated Diseases

- Autoimmune diseases: e.g. rheumatoid arthritis, Sjögren's syndrome, systemic lupus erythematosus, inflammatory bowel disease.
- Immune-mediated disease: e.g. allergic bronchopulmonary aspergillosis.

Obstructive Bronchiectasis

It is **localized** to the obstructed segment of the lung.

Airway/Bronchial Obstruction

- **Partial or total obstruction of the bronchial lumen:** It may be caused by tumor, foreign body aspiration and mucus plugs. It may also develop in patients with atopic asthma and chronic bronchitis.

Cystic Fibrosis

Major respiratory diseases in cystic fibrosis (CF) are sinusitis and bronchiectasis.

Pathogenesis

It is **complex process**. Both **obstruction and chronic persistent infection** are the two major etiological conditions seem to be necessary for the development of bronchiectasis.

1. **Obstruction:** It **impairs clearing mechanisms** of the lung → results in **accumulation of secretions** distal to the obstruction → **leads to secondary infection** → inflammation → weakens and dilates airway.
2. **Chronic persistent necrotizing infection:** Chronic infection in the bronchi or bronchioles → increased bronchial secretion → **obstruction** of airways by secretions → inflammation and fibrosis of the airway walls → weakening and dilatation of airways.

Bronchiectasis associated with cystic fibrosis: It shows the **importance of both infection and obstruction**. In cystic fibrosis there is defect in the mucociliary action and there is accumulation of thick viscid secretions → leads to obstruction of the airways → favors microbial infections. With repeated infections the walls of airway are damaged and lead to dilatation of bronchi and bronchioles.

In patients with asthma and cystic fibrosis hypersensitivity reaction to the fungus may cause allergic bronchopulmonary aspergillosis. This may predispose to proximal bronchiectasis.

Bronchiectasis:

1. Obstruction
2. Chronic persistent necrotizing infection.

MORPHOLOGY

Classification

Q. Write short note on gross and microscopic pathology of bronchiectasis.

A. According to the shape of the bronchial dilation (Fig. 16.7).

1. **Saccular (cystic) bronchiectasis:** It is characterized by markedly dilated bronchi which end blindly in dilated sacs.
2. **Cylindrical (tubular) bronchiectasis:** It shows uniform and moderate dilation.
3. **Fusiform:** Characterized by fusiform dilatation.
4. **Varicose bronchiectasis:** It shows irregular dilations and constrictions. They resemble varicose veins when seen by radiologic bronchography.

B. According to the extent of involvement

1. **Generalized:** It is **usually bilateral** and commonly affects the **lower lobes. Left lobe is more commonly involved than the right.** It is most severe in the more distal bronchi and bronchioles.
2. **Localized:** It is **restricted to a single segment of the lung** and usually occurs in association with obstruction (tumors or aspiration of foreign bodies).

Gross (Fig. 16.8)

Normal lung: Bronchioles cannot be traced by ordinary gross dissection beyond a point 2–3 cm from the pleural surfaces.

Bronchiectasis: Dilated airways can be traced up to the pleura.

- **Bronchi and bronchioles are dilated and can be traced almost up to the pleural surfaces.**
- **Extent of dilatation:** It may be up to four times normal lumen/size.
- **Cut surface:** The **dilated bronchi and bronchioles** appear as saccular, cylindrical or varicose (depending on the type). The **wall of the bronchi is thickened** by fibrosis. The **lumen is filled with thick, mucopurulent secretions** and the mucosal surface is congested.

Microscopy (Fig. 16.9)

Varies with the activity and chronicity of the disease.

Bronchial Wall

- **Dilatation of bronchi and bronchioles** and destruction of their wall due to severe inflammation. If severe → may lead to lung abscess.
- **Dense infiltration of chronic inflammatory cells:** It mainly consists of **lymphocytes and plasma cells** within the **walls of the bronchi and bronchioles**. Sometimes, prominent lymphoid follicles may be seen.
- **Fibrosis of the bronchial and bronchiolar wall:** It occurs in the chronic cases → may result in partial or complete obliteration of bronchiolar lumens.

Mucosal Changes

- **Desquamation of the lining epithelium** and areas of ulceration is seen.
- **Lining columnar cells** may show **pseudostratification** and squamous **metaplasia in the surrounding epithelium**.

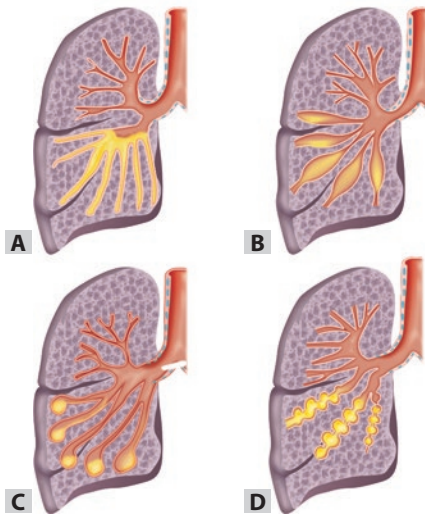
Microbes: Culture of content from the involved bronchi may show **mixed microbes**. These include staphylococci, streptococci, pneumococci, enteric organisms and (particularly in children) *Haemophilus influenzae* and *Pseudomonas aeruginosa*.

In allergic bronchopulmonary aspergillosis fungal hyphae can be seen on special stains.

Q. Write short note on complications of bronchiectasis.

Complications:

1. Lung abscess
2. Pneumonia
3. Empyema
4. Septicemia
5. Meningitis
6. Metastatic abscesses (e.g. brain)
7. Cor pulmonale
8. Generalized amyloidosis.



Figs 16.7A to D: Morphological types of bronchiectasis: (A) Cylindrical type; (B) Fusiform type; (C) Saccular; (D) Varicose type

Clinical Course

- **Severe persistent productive cough:** It is the most common clinical feature.
- **Sputum is foul-smelling**, sometimes bloody. In severe cases, the patient presents with dyspnea and orthopnea.
- Symptoms are precipitated by upper respiratory tract infections.
- **Paroxysms of cough** occur when the patient rises in the morning. This is because, the changes in position lead to drainage of collections of pus and secretions into the bronchi.

PULMONARY INFECTIONS

Respiratory tract infections are the most frequent infections.

Causes

- **Upper respiratory tract infections:** Majority are caused by viruses (common cold, pharyngitis).
- **Lower respiratory tract infections:** It may be due to bacteria, virus, mycoplasma and fungus.

PNEUMONIA

Definition: Pneumonia is defined as **infection of the lung parenchyma**. It causes the alveoli to be filled with inflammatory exudates and usually results in consolidation (solidification) of lung.

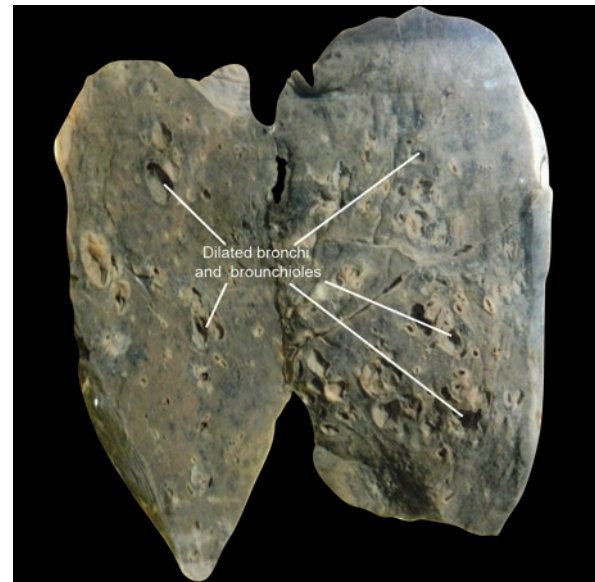
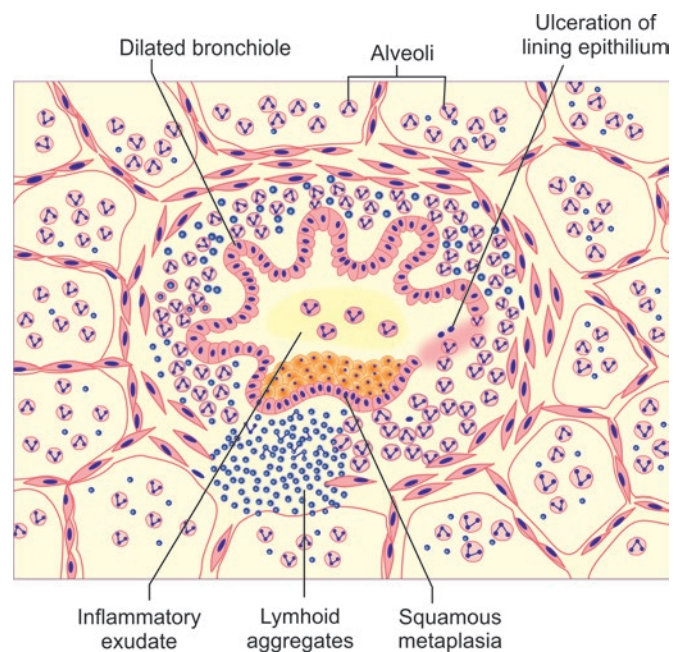


Fig. 16.8: Gross appearance of bronchiectasis with dilated bronchi and bronchioles



Microscopy of bronchiectasis: Bronchi and bronchioles show

- Dilatation
- Dense chronic inflammation
- Desquamation of lining epithelium
- Squamous metaplasia
- Fibrosis.

Fig. 16.9: Microscopic features of bronchiectasis (diagrammatic)

Etiology

Q. Etiology and pathogenesis of lobar pneumonia.

Factors Favoring Development of Pneumonia

- **Lowered systemic resistance of the host:** It may be due to
 - **Chronic diseases.**
 - **Immunological deficiency:**
 - ◆ Defects in innate immunity and humoral immunodeficiency.
 - ◆ Defects in cell-mediated immunity (congenital and acquired).
 - **Treatment with immunosuppressive agents.**
 - **Leukopenia.**
- **Impaired local defense mechanisms**
 - **Loss or suppression of the cough reflex:** e.g. coma, anesthesia, drugs, chest pain, or neuromuscular disorders. It may lead to aspiration of gastric contents into the lung.
 - **Damage or injury to the mucociliary apparatus:** It may be due to cigarette smoke, viral diseases, inhalation of hot or corrosive gases, or genetic defects of ciliary function (e.g. the immotile cilia syndrome).
 - **Accumulation of secretions:** Cystic fibrosis and bronchial obstruction.
 - **Interference with the phagocytic or bactericidal action of alveolar macrophages:** It may be due to alcohol intoxication, tobacco smoke, anoxia, or oxygen intoxication.
 - **Pulmonary congestion and edema.**

Pneumonia:

1. Lowered systemic resistance of host
2. Impaired local defense mechanism.

Portal of Entry

The causative agents may enter the lung parenchyma by following routes:

1. Through respiratory tract.
2. Hematogenous spread.
3. Local spread.

Classification of Pneumonia (Box 16.2)

For classification of pneumonia, see Box 16.2.

COMMUNITY-ACQUIRED ACUTE PNEUMONIAS

Community-acquired pneumonia: Commonest cause is *Streptococcus pneumoniae*.

BOX 16.2: Classification of pneumonia

1. Classification depending on the anatomic distribution
 - Lobar pneumonia
 - Bronchopneumonia
 - Interstitial pneumonia
2. Etiological classification
 - Primary
 - Secondary
 - Suppurative
3. Clinical setting in which the infection occurs (if no pathogen can be isolated)
 - Community-acquired acute pneumonia
 - Community-acquired atypical pneumonia
 - Nosocomial pneumonia or hospital-acquired pneumonia
 - Pneumonia in immunocompromised host
 - Health-care associated pneumonia

Most common cause of atypical pneumonia: *Mycoplasma pneumoniae*

Nosocomial pneumonia: Commonest cause is *Staphylococcus aureus*.

TABLE 16.1: Causative agents and salient features of community-acquired acute pneumonia

Microorganism	Features
<i>Streptococcus pneumoniae</i> or <i>Pneumococcus</i>	Most common cause
<i>Haemophilus influenzae</i>	Most common bacterial cause in COPD
<i>Moraxella catarrhalis</i>	Elderly
<i>Staphylococcus aureus</i>	Secondary bacterial pneumonia following viral respiratory illnesses
<i>Enterobacteriaceae</i> (<i>Klebsiella pneumoniae</i>)	In debilitated and malnourished people
<i>Pseudomonas aeruginosa</i>	Common in patients with neutropenia
<i>Legionella pneumophila</i>	Organ transplant recipients

Definition: Community-acquired pneumonia includes infectious pneumonia in patients living independently in the community.

Causative Microorganisms (Table 16.1)

These may be bacteria or virus. Bacterial infection of the lung parenchyma **causes the alveoli to be filled with an inflammatory exudate and produces consolidation** (solidification) of the pulmonary parenchyma.

Predisposing Conditions

- **Extremes of age.**
- **Chronic diseases:** e.g. congestive heart failure, COPD and diabetes.
- **Congenital or acquired immune deficiencies.**
- **Decreased or absent splenic function:** e.g. sickle cell disease or post-splenectomy (risk for infection with encapsulated bacteria).

Pathogenesis

Various mechanisms by which lung is infected are as follows:

- **Aspiration of oropharyngeal contents:** Most frequent.
- **Inhalation of aerosolized droplets:** Second most frequent.
- **Blood stream infection:** Less common.

Classification of Bacterial Pneumonia

Depending on the anatomic distribution, bacterial pneumonia can be divided into lobular bronchopneumonia and lobar pneumonia.

- **Lobar pneumonia:** It is defined as fibrinosuppurative consolidation of a large portion of a lobe or of an entire lobe of the lung (Fig. 16.10A).
- **Bronchopneumonia:** It is characterized by patchy (scattered solid foci) area consolidation in the same or several lobes of the lung (Fig. 16.10B).

These two anatomic patterns may overlap in the same lung. The same organisms may produce either pattern, which depends on the susceptibility of the patient.

Bacterial pneumonia: From the clinical standpoint, it is very important to identify the causative agent and determine the extent of disease.

LOBAR PNEUMONIA

Q. Gross and microscopic features of lung in lobar pneumonia.

Q. Stages and gross appearance of lobar pneumonia.

Lobar pneumonia: Most common cause is *Streptococcus pneumoniae* or pneumococcus

Lobar pneumonia is characterized by **diffuse inflammation** affecting the **part or entire lobe**, usually the lower lobes (Fig. 16.10A).

MORPHOLOGICAL STAGES

The inflammatory response is classically divided into four stages: Congestion, red hepatization, gray hepatization and resolution.

Lobar pneumonia: Four stages

1. Congestion
2. Red hepatization
3. Gray hepatization
4. Resolution.

Congestion

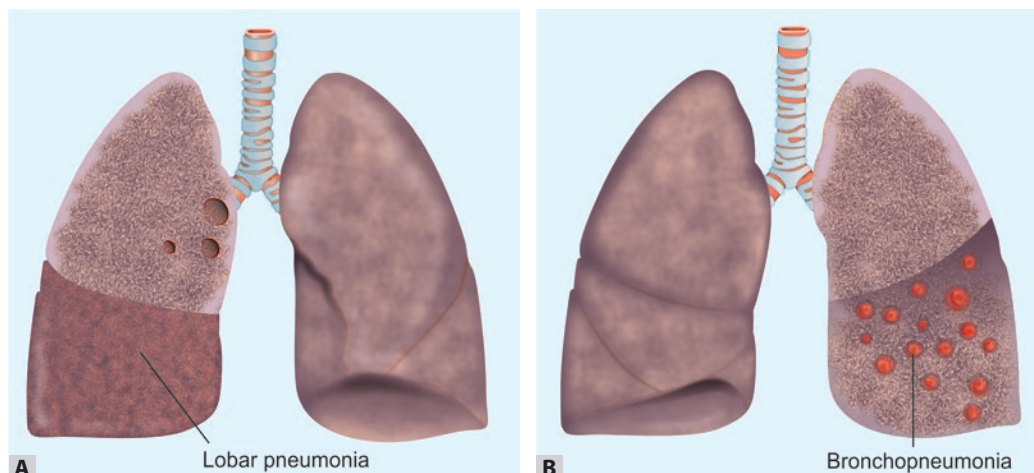
This stage lasts for less than 24 hours.

- **Gross:** The involved lobe of the lung is **heavy, boggy and red**. A **blood-stained frothy fluid oozes from the cut surface**.
- **Microscopy** (Fig. 16.11A): It is characterized by
 - **Dilatation and congestion of capillaries** in the alveolar walls.
 - The air spaces in the alveoli are filled with **pale eosinophilic fluid with few neutrophils, red cells and numerous bacteria**.

Red Hepatization

It lasts for 2–3 days.

- **Gross:** The lobe appears **distinctly red, firm and airless**. The firm consistency of the affected lobe of the lung resembles that of the liver, this stage has been aptly termed **red hepatization**. The pleura may show **serofibrinous pleurisy**. Cut section shows **red, and granular appearance**.



Figs 16.10A and B: (A) Lobar pneumonia, anatomically involves whole or part of a lobe; (B) Bronchopneumonia, anatomically shows patchy area of consolidation

- **Microscopy** (Figs 16.11B and 16.12):
 - Alveoli show **exudate** and interlacing strands of fibrin (formed from clotted fibrinogen present in fluid exudate)
 - **Numerous neutrophils** and red cells are found in the fibrin meshwork.

Gray Hepatization

Q. Write short note on gray hepatization.

Gray hepatization of lungs is seen on day: 5–7 days.

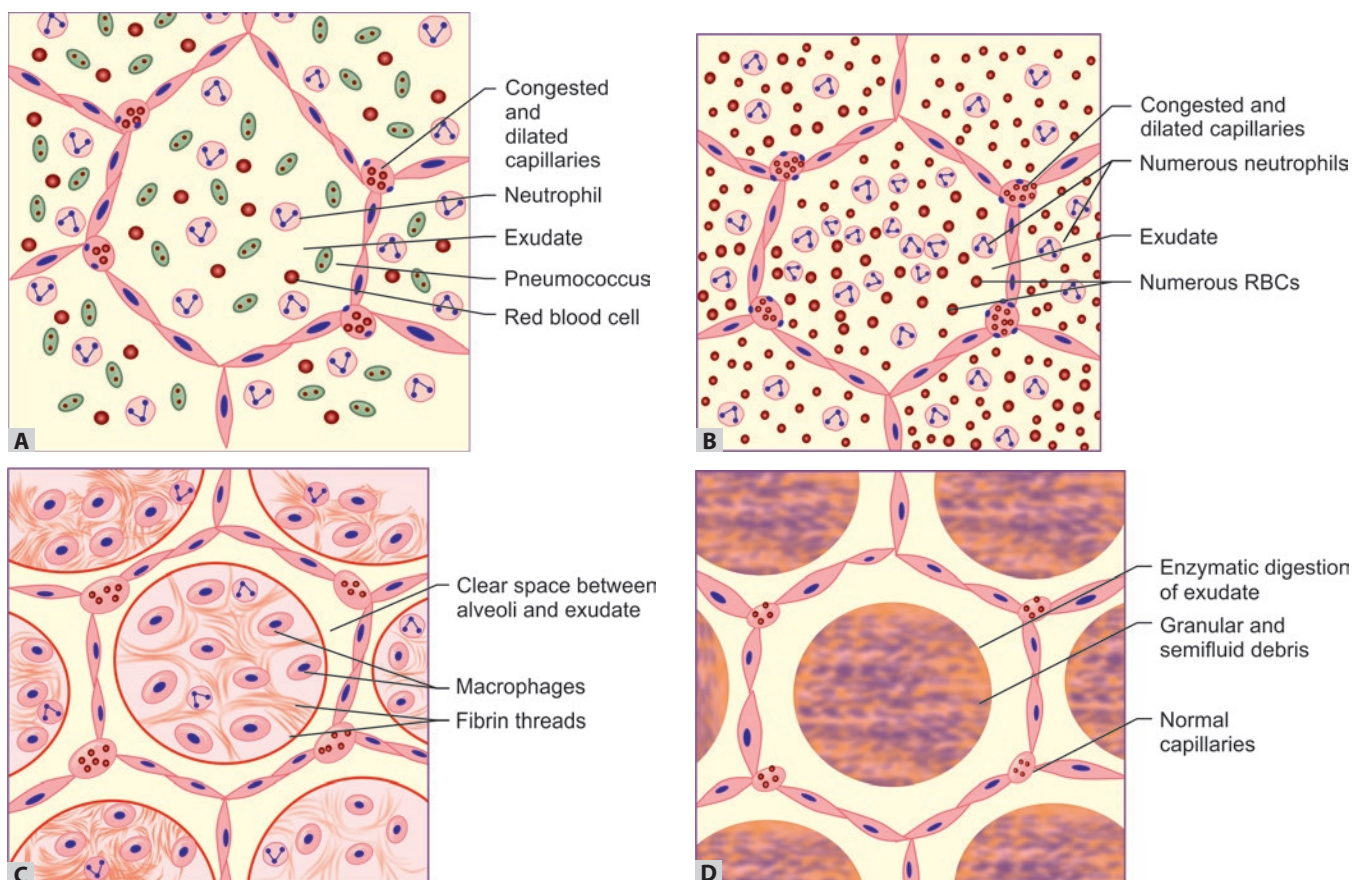
- **Gross** (Fig. 16.13): The affected lobe gradually loses its red color and assumes gray appearance. Cut surface shows gray, dry and granular appearance.
- **Microscopy** (Figs 16.11C and 16.14):
 - Progressive disintegration of red cells.
 - **Fibrinosuppurative exudate** in the alveoli in which neutrophils are replaced by macrophages.
 - There is a **clear space between the alveolar wall and the exudate** due to contraction of the fibrin thread (formed from fibrinogen in the exudate) present in the exudate.

Resolution

Resolution occurs by **liquefaction** of the previously solid, fibrinous constituents of the exudate in the air spaces. The fibrinous exudate within the air spaces undergoes progressive liquefaction to produce granular and semifluid debris. The liquefaction is due to fibrinolytic enzymes liberated from neutrophils. The liquefied material is removed, partly by expectoration, but mainly ingested by macrophages and drained through lymphatics. If not removed, it may become organized by ingrowth of capillaries and fibroblasts into the exudate.

- **Gross**: The affected lobe becomes more **crepitant** as the air spaces reopen. Cut section appears frothy.
- **Microscopy** (Fig. 16.11D): The alveolar space shows granular material and semifluid debris.

Bronchopneumonia: Acute bronchitis with extension into adjacent alveoli.



Figs 16.11A to D: Microscopic features of different stages of lobar pneumonia (diagrammatic). (A) Congestion; (B) Red hepatization; (C) Gray hepatization; (D) Resolution

BRONCHOPNEUMONIA**Q. Write short note on bronchopneumonia.**

Bronchopneumonia: Usually

1. Bilateral basal location due to gravitation of secretions
2. Affects extremes of age (infants or old)
3. X-ray chest—patchy opacification of the lobe.

Gross (Fig. 16.10B)

- Bronchopneumonia is characterized by **widespread focal/patchy areas of acute suppurative inflammation**.
- They are **centered on bronchioles and bronchi** with subsequent spread to surrounding alveoli. The involved alveoli show **consolidation**.
- The **consolidated areas larger and more numerous in lower lobes** (because of the tendency of secretions to gravitate into the lower lobes) and **frequently bilateral**.

Q. Differences between bronchopneumonia and lobar pneumonia.

- **Cut section:** It appears slightly raised above the surface of the surrounding lung and measure several millimeters in diameter. They are **dry, solid, granular, gray-red to yellow, and poorly delimited at their margins**.

Microscopy

The bronchi, bronchioles, and adjacent alveolar spaces are filled exudate rich in neutrophils (Fig. 16.15).

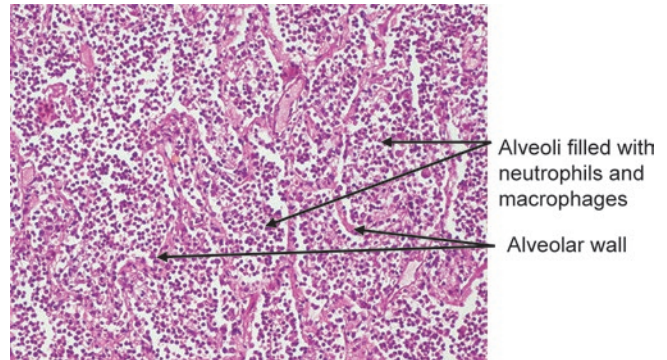


Fig. 16.12: Red hepatization in lobar pneumonia (H and E)

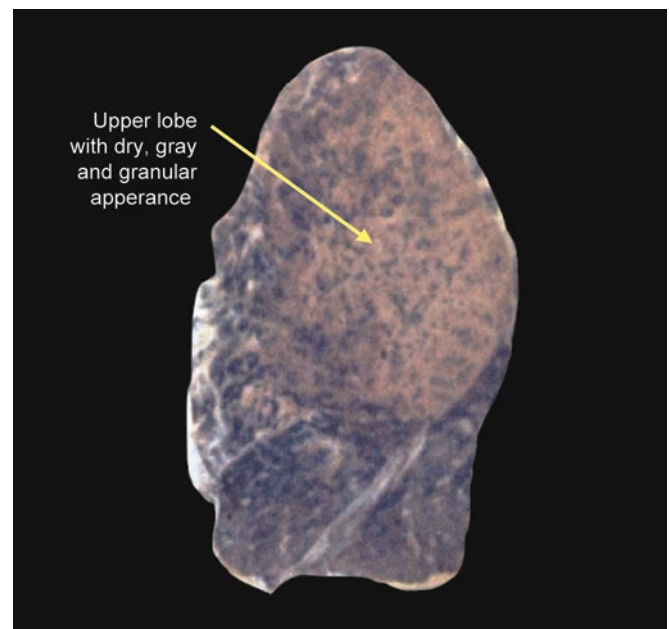


Fig. 16.13: Gross appearance of gray hepatization of lung

Complications of Pneumonia**Q. Write short note on complications of pneumonia.**

1. **Lung abscess:** It may develop with extensive tissue destruction and necrosis.
2. **Organization:** Delayed and incomplete resolution can cause **ingrowth of granulation tissue into the alveolar exudate**. The intra-alveolar plugs of granulation tissue are known as organizing pneumonia. Gradually, **increased alveolar fibrosis leads to a shrunken and firm lobe and is called as carnification**.
3. **Spread of infection to the pleural cavity:** It may result in:
 - **Pleuritis** (inflammation of the pleura) due to extension of inflammation from lung. It may resolve or more often undergoes organization, and fibrous thickening or permanent adhesions.
 - **Pleural effusion** (fluid in the pleural space) is common and usually resolves.
 - **Pyothorax (pus in the pleural space):** It results from an infection of a pleural effusion and may heal with extensive fibrosis.
 - **Empyema:** It is a loculated collection of pus (intrapleural fibrinosuppurative reaction) with fibrous walls and may result from the persistence of pyothorax.

4. **Bacteremic dissemination (bacteremia):** It can cause:
 - Endocarditis (heart valves).
 - Pericarditis (pericardium).
 - Meningitis (meninges).
 - Suppurative arthritis (joint).
 - Metastatic abscesses in kidneys or spleen.

Complications of pneumonia:

1. Lung abscess
2. Organization
3. Spread of infection to the pleura
4. Bacteremic dissemination.

Clinical Feature

- Sudden onset of high fever, chills and coughs with mucopurulent sputum.

TABLE 16.2: Differences between lobar pneumonia and bronchopneumonia

Feature	Lobar pneumonia	Bronchopneumonia
Definition	Diffuse inflammation affecting the part or the entire lobe/s (usually lower lobes)	Widespread focal/ patchy area of acute suppurative infection of terminal bronchioles extending into adjoining alveoli
Age group affected	More common in adults	Commonly develops at extremes of age (infants and old age)
Predisposing factors	Usually develops in healthy individuals	Usually accompany pre-existing diseases (e.g. chronic debility influenza)
Common causative organisms	Pneumococci, <i>Klebsiella pneumoniae</i> staphylococci, streptococci	Staphylococci, streptococci, <i>Pseudomonas</i> , <i>Haemophilus influenzae</i>
Pathologic features	Usually passes through four stages namely (i) congestion, (ii) red hepatization, (iii) gray hepatization and (iv) resolution	Patchy consolidation. Bronchi, bronchioles and adjacent alveolar spaces are filled with exudate rich in neutrophils
Blood findings	Neutrophilic leukocytosis and positive blood culture	Neutrophilic leukocytosis, positive blood culture
X-ray	Shows consolidation	Shows mottled focal opacities
Complications	Less common and include pleural effusion, empyema, lung abscess	Bronchiectasis and complications similar to lobar pneumonia
Response to treatment	Better	Variable
Prognosis	Good	Poor

- Fibrinosuppurative pleuritis causes pleuritic pain and accompanied by pleural friction rub.
- Radiography: Involved lobe appears radiopaque in lobar pneumonia and focal opacities in bronchopneumonia.

X-ray chest: Gold standard for diagnosing pneumonia.

Positive Gram stain on sputum: More useful than culture.

Differences between lobar pneumonia and bronchopneumonia (Table 16.2).

Q. Differences between lobar pneumonia and bronchopneumonia.

Pneumocystis Pneumonia

Q. Write short answer on Pneumocystis pneumonia.

- Pneumocystis pneumonia is an opportunistic fungal infection of the lungs by inhalation of *Pneumocystis jirovecii*.
- It usually infects neonates and immunosuppressed individuals (HIV/AIDS, immunosuppression by chemotherapy for organ transplant and tumors, malnutrition, agammaglobulinaemia, etc.). In HIV, *Pneumocystis pneumonia* usually develops when the CD4⁺ count is less than 200 cells/mm³.

Morphologic Features

Gross

The affected parts of the lung are consolidated, dry and grey.

Microscopy

- Interstitial pneumonitis with thickening and mononuclear infiltration of the alveolar walls.
- Alveoli contain **pink frothy fluid** with the organisms. On staining by **Grocott's methenamine-silver (GMS) stain**, they appear as oval or crescentic cysts, about 5 µm in diameter and **surrounded by numerous tiny black dotlike organism**. *P. jirovecii* are demonstrable in the frothy fluid.
- No significant inflammatory reaction in the air spaces.

Clinical Course

- Rapid onset of dyspnea, tachycardia, cyanosis and non-productive cough. Chest x-ray shows diffuse alveolar and interstitial infiltrate.
- If untreated, death occurs in one or two weeks.

Community: Acquired Viral Pneumonia

Q. Write short answer on community-acquired pneumonia.

Etiology

- Viruses that cause pneumonia include influenza virus types A and B, the respiratory syncytial viruses, human metapneumovirus, adenovirus, rhinoviruses, rubeola and varicella viruses.

Pathogenesis

- Viruses can cause a mild upper respiratory tract infection or a more severe lower respiratory tract infection.
- **Predisposing factors** for extension of the infection into the lower respiratory tract include extremes of age, malnutrition, alcoholism and underlying debilitating illnesses.

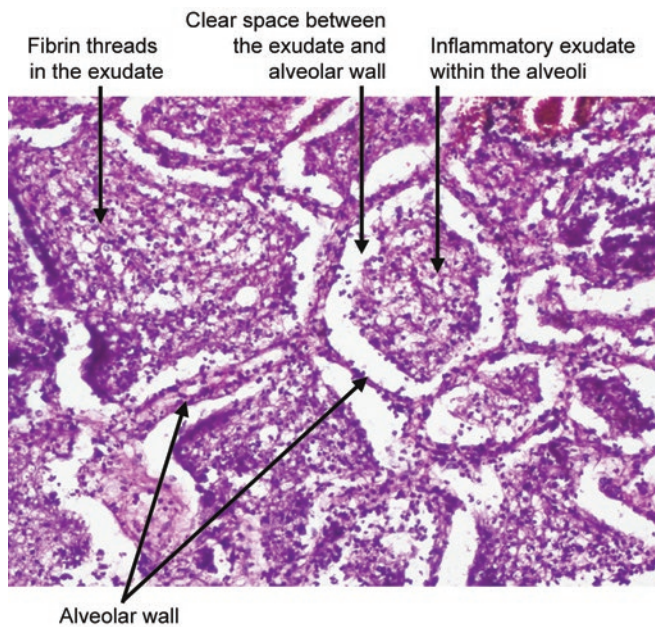


Fig. 16.14: Gray hepatization in lobar pneumonia (H & E)

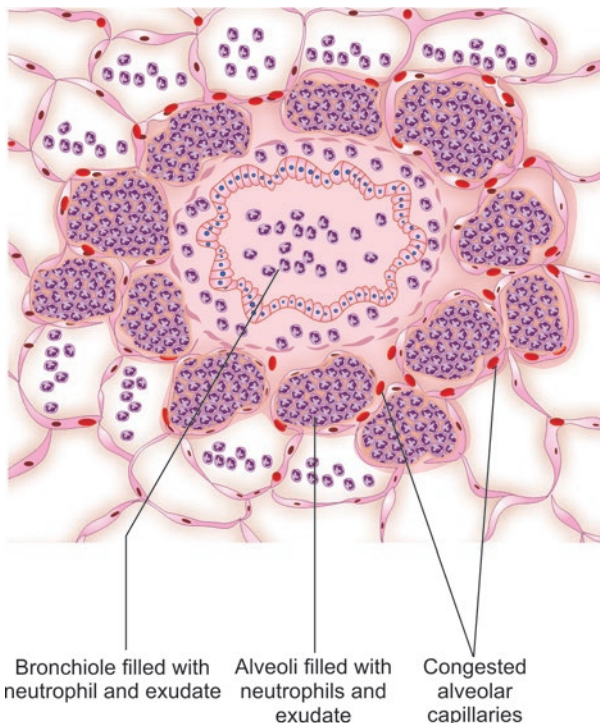


Fig. 16.15: Bronchopneumonia characterized by infiltration of bronchi, bronchioles and adjacent alveoli by acute inflammatory exudate

- **Damage to respiratory lining cells:** These viruses have affinity to attach and enter respiratory lining cells. Viral replication leads to cytopathic changes, leading to death of cells and secondary inflammation.
- **Secondary bacterial infection:** The damage produced by virus impairs the local pulmonary defensive forces (e.g. mucociliary clearance) and predispose to superinfection by bacteria. Thus, secondary bacterial infection usually becomes more serious than the viral infection itself.

MORPHOLOGY

Gross

Lung may show either patchy or entire lobes bilaterally or unilaterally. The affected region appears red-blue and congested. Pleuritis or pleural effusions are infrequent.

Microscopy

Depends on the severity of the disease.

- **Interstitial inflammatory reaction:** Involving the walls of the alveoli is the **predominant feature**. The alveolar septa shows widening, edema and mononuclear inflammatory infiltrate composed of lymphocytes, macrophages, and occasionally plasma cells. In acute cases neutrophils may also be seen.
- The alveoli may show intra-alveolar proteinaceous material and a cellular exudate. If patient develops ARDS, pink hyaline membranes may form lining of the alveolar walls.
- Eradication of the viral infection results in resolution and return of the normal lung architecture.
- If there is superimposed bacterial infection, ulcerative bronchitis, bronchiolitis and bacterial pneumonia may develop.

Clinical Course

Extremely varied. Many present as severe upper respiratory tract infections with fever, headache, muscle aches and pains in the legs. Cough may be absent. Usually viral infections are mild and resolve spontaneously without any sequelae. However, interstitial viral pneumonias may assume epidemic proportions, and can lead to morbidity and mortality.

HOSPITAL-ACQUIRED PNEUMONIA

Definition: Hospital-acquired pneumonias are pulmonary infections acquired in the course of a hospital stay.

Etiology

Predisposing factors: Severe underlying disease, immunosuppression, prolonged antibiotic therapy, patients on

BOX 16.3: Various etiological agents causing hospital-acquired pneumonia

Gram-negative bacteria, Enterobacteriaceae (*Klebsiella* species *Serratia marcescens*, *Escherichia coli*) and *Pseudomonas* species.

Staphylococcus aureus (penicillin resistant).

Atypical pneumonia:

- Interstitial pneumonia
- No signs of consolidation.

mechanical ventilation or invasive access devices, such as intravascular catheters. The hospital-acquired pneumonias are serious and may be life threatening.

The common causative organism causing hospital-acquired pneumonia are shown in Box 16.3.

LUNG ABSCESS

Q. Write short note on lung abscess.

Definition: Lung (pulmonary) abscess is a **local suppurative process within the lung**. It is characterized by **accumulation of pus accompanied by the destruction of lung tissue**.

Etiology and Pathogenesis

Lung abscess:

Local suppurative process within the lung.

Causative Organisms

- Any pathogen can produce lung abscess.
- It is usually due to aerobic and anaerobic organisms normally found in the oral cavity (gram-negative organisms).
- Mixed infections occur when lung abscess develops due to inhalation of foreign material.
- **Causative agents:** Anaerobic bacteria (extremely common), with or without mixed aerobic infection, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus pyogenes* and type 3 *Pneumococcus* (uncommon).

Lung abscess: Commonest etiological agent anaerobic bacteria.

Mechanism

Lung abscess may be **primary or secondary**.

Lung abscess:

- Primary
- Secondary.

A. Secondary Lung Abscess

Develops as a complication of several conditions.

- **Complication of necrotizing pneumonia:** The microbes usually associated are as follows: *S. aureus*, *K. pneumoniae*, and the type 3 *Pneumococcus*.
- **Septic embolism:** The source of embolus may be from thrombophlebitis in any part of the systemic venous circulation or from the vegetations of infective bacterial endocarditis on the right side of the heart. The embolus may be trapped in the lung causing multiple pyemic abscesses.
- **Bronchial obstruction:** By a **bronchial cancer** (primary or secondary) or foreign body (postobstructive pneumonia) → secondary infection.
- **Miscellaneous:**
 - Direct penetrating trauma to the lungs.
 - Spread of infections from a neighboring organs (e.g. suppuration in the esophagus, spine, subphrenic space, or pleural cavity).

B. Primary Cryptogenic Lung Abscesses

These type of lung abscess have no apparent cause and most of them develop as consequence of **aspiration of infected material**. Bacteria responsible are usually the mixed anaerobes found in the oropharynx.

- **Aspiration of infective material:** From **infected nasal sinuses or tonsils** or periodontitis gingivitis may occur during alcoholic stupor, general anesthesia, coma, head injury or neurological disease causing loss of consciousness.
- **Aspiration of gastric contents.**

Lung abscess: Commonest cause is aspiration.

MORPHOLOGY

Gross (Fig. 16.16)

1. Site and number:

- Lung abscess may develop **any part of the lung** and may be **single or multiple**.
- Abscess due to **aspiration** are more common on the **right** (because of the more vertical right main bronchus) and are most often **single**.
- Abscesses that complicate **pneumonia or bronchiectasis** are usually **multiple, basal, and diffusely scattered**.
- **Septic emboli** and pyemic abscesses are **multiple** and may affect **any region** of the lungs.

2. Size: Vary from few millimeters to large cavities of 5–6 cm.

3. Content:

- Abscess cavity may contain **suppurative debris**.
- If **communication** with an air passage → the abscess may be partially drained to produce an **air-containing cavity**.
- **Continued infection** may result in → **gangrene of the lung**.

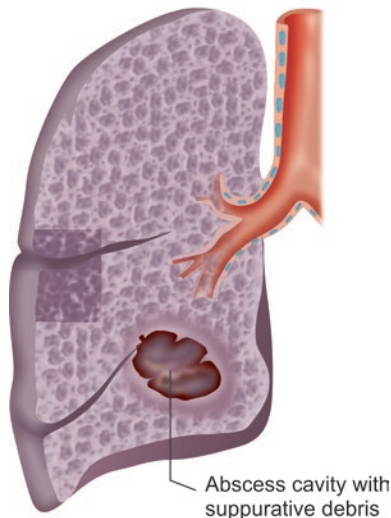


Fig. 16.16: Lung abscess with suppurative abscess (diagrammatic)

Microscopy

- **Cardinal feature:** It is characterized by suppurative destruction of the lung parenchyma within the central area of cavitation.
- Abscess shows numerous polymorphonuclear leukocytes and variable numbers of macrophages. The abscess is surrounded by fibrous wall infiltrated by inflammatory cells.

Clinical Course

- Similar to bronchiectasis and is characterized by cough, fever, and large amounts of foul-smelling purulent or sanguineous sputum.
- Fever, chest pain, and weight loss are common.
- Clubbing of the fingers and toes may appear. Lung abscess in older individuals may be associated with carcinoma.

Complications

1. Extension of the infection into the pleural cavity leading to empyema/pneumothorax/bronchopleural fistula.
2. Hemorrhage into the abscess cavity.
3. Septic emboli may cause brain abscesses or meningitis.
4. Secondary amyloidosis (type AA).

TUBERCULOSIS

Tuberculosis: Communicable, chronic granulomatous disease caused by *Mycobacterium tuberculosis*.

Tuberculosis (also called Koch's disease) is a communicable, chronic granulomatous disease caused by *Mycobacterium tuberculosis*.

- Most cases of tuberculosis are caused by *Mycobacterium tuberculosis hominins* (human strain). The source of infection is patients with active open case of tuberculosis.
- **Oropharyngeal and intestinal tuberculosis** can be caused by drinking milk contaminated with *M. bovis* (bovine strain) from infected cows. Routine pasteurization has almost eliminated this source of infection.
- *M. avium* and *intracellulare*, which are nonpathogenic to normal individuals but cause infection in patients with AIDS.

Characteristics of Mycobacteria

- It is an aerobic, slender, rod-shaped bacteria measuring 2–10 μm in length.
- It has a lipid coat which makes it difficult to stain, but once stained resists decolorization by acids and alcohol. Hence, it is termed as acid-fast bacilli (AFB), because once stained by carbol fuchsin (present in Ziehl-Neelsen stain), it is not decolorized by acid and alcohol.

Acid-fastness of the *Mycobacterium* is due to mycolic acid.

Ziehl-Neelsen stain: Acid-fast bacilli (AFB) stain for demonstration of mycobacteria.

Epidemiology

Active tuberculosis: Develops only in few individuals who get infection.

- Tuberculosis is common in India. Incidence of tuberculosis is high wherever there is poverty, overcrowding and chronic debilitating illness.
- Diseases are associated with increased risk: Diabetes mellitus, Hodgkin lymphoma, malnutrition, immunosuppression, alcoholism, chronic lung disease (e.g. silicosis) and chronic renal failure.
- HIV is the most important risk factor.

Infection Versus Disease

In most, primary tuberculosis is asymptomatic but when immune defenses are lowered, the infection may produce potentially life-threatening disease.

Infection with *M. tuberculosis* is to be differentiated from disease.

- **Infection:** Tuberculous infection indicates the presence of organisms in a person, which may or may not cause symptomatic disease.
- **Disease:** Active tuberculosis refers to subset of tuberculous infections manifested by destructive, symptomatic disease.

Mode of Transmission

Mode of transmission of tuberculosis:

1. Inhalation
2. Ingestion
3. Inoculation (very rarely).

Inhalation

- It is the **most common mode** of transmission.
 - **Source of organisms** is from an **active open case of tuberculosis** to a susceptible host.
 - Tubercle bacilli will be expelled while coughing, sneezing that creates aerosolized respiratory droplets. When an individual inhales the droplets, it lodges in the lung and causes infection.

Open case of tuberculosis is a patient with tuberculosis in which cavitary lung lesion is communicated to the external environment through the patient's airways.

Pulmonary tuberculosis: Spread by droplet infection.

Ingestion

- Tuberculosis may be transmitted by drinking nonpasteurized milk from infected cows contaminated with *Mycobacterium bovis*.
- Site of infection: *Mycobacterium bovis* causes oropharyngeal and intestinal tuberculosis.
- Eradication of tuberculous herds with tuberculosis and **pasteurization** has almost **eliminated this mode of transmission** of tuberculosis. Nowadays, the ingestion mode of transmission occurs when a patient with open case of tuberculosis swallows the infected sputum which results in tuberculosis of intestine.

Tuberculosis: Reservoir of infection is patient with active tuberculosis.

Inoculation

- It is **extremely rare mode** of transmission. May develop during postmortem examination, while cuts resulting from handling tuberculous infected organs.

Tuberculin (Mantoux) Test

Tuberculin test positivity indicates: Good cell-mediated immunity

- Infection by mycobacteria → development of delayed hypersensitivity to *M. tuberculosis* antigens → which is detected by the tuberculin skin test.

- About 2–4 weeks after infection, **intracutaneous injection of 0.1 mL of purified protein derivative (PPD)** of *M. tuberculosis* produces a **visible and palpable induration of 10 mm diameter or more** at the site of injection of PPD. The induration peaks in 48–72 hours.
- **Significance:**
 - **Positive tuberculin test:** It indicates **T cell-mediated immunity** to mycobacterial antigens.
 - **False-negative reactions:** It is seen in certain viral infections, sarcoidosis, malnutrition, Hodgkin lymphoma, immunosuppression and overwhelming active tuberculous disease.
 - **False-positive reactions:** It is seen in infection by **atypical mycobacteria** or prior **vaccination with BCG** (*Bacillus Calmette-Guerin*).

Positive Mantoux test indicates that the individual is exposed to mycobacterial antigen. It does not differentiate between infection and disease.

Pathogenesis

Q. Pathogenesis of tuberculosis.

Immunity and hypersensitivity: Infection with tubercle bacillus results in two simultaneous immunological responses.

- Cell-mediated immunity.
- Type IV hypersensitivity reaction.

The effector cells for both cell-mediated immunity and hypersensitivity reaction are T_H1 cells.

- **Cell-mediated immunity:** Tuberculosis developing first time in an immunocompetent individual depends on the cell-mediated immunity.
- **Type IV hypersensitivity reactions:** Develops to mycobacterial antigens and is **responsible for the tissue destruction, such as caseating granulomas and cavitation**. Appearance of type IV hypersensitivity reaction also signals the development of protective immunity.

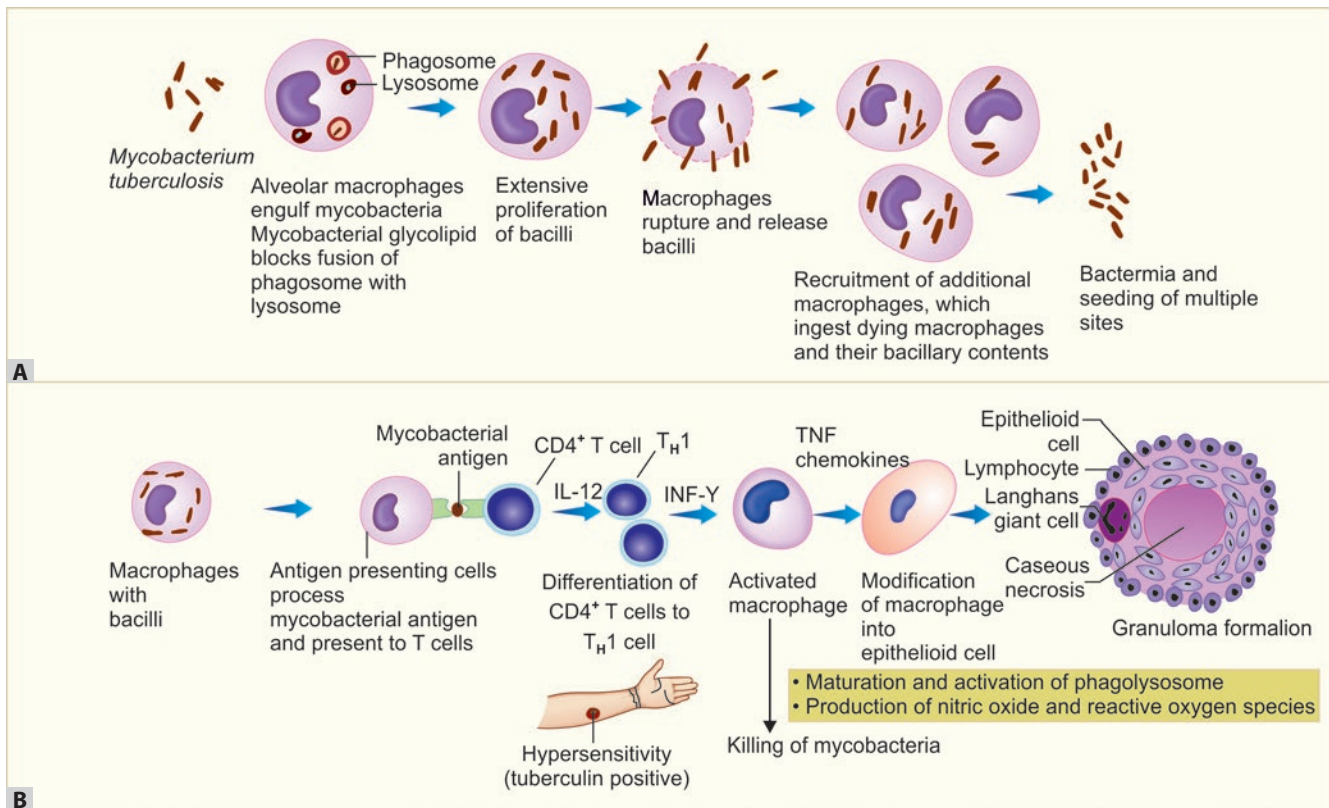
In tuberculosis, immunity (resistant to infection) and hypersensitivity are two different manifestations of same mechanism.

Tuberculosis: Usually affect lungs, but any organ may be involved.

Mechanism of Granuloma Formation in Tuberculosis (Fig. 16.17)

The granuloma in tuberculosis is known as tubercle.

Tuberculosis is a chronic granulomatous disease, caused by *M. tuberculosis*. Lung being commonly involved in



Figs 16.17A and B: Sequence of events in primary tuberculosis of lung: (A) Infection before activation of cell-mediated immunity; (B) Initiation and consequences of cell-mediated immunity

tuberculosis, the pathogenesis is considered with respect to pulmonary tuberculosis.

Infection before Activation of Cell-mediated Immunity

Initial infection with *Mycobacterium tuberculosis* in a nonsensitized individual is called **primary tuberculosis**. The following sequence of events occur:

Phagocytosis of mycobacteria by macrophages:

First time, when the **virulent tubercle bacilli** are deposited in the tissue, they primarily **infect macrophages**. In the lung, they **undergo endocytosis** into the alveolar macrophages through **macrophage receptors**. These receptors include:

- **Mannose receptors** bind lipoarabinomannan (LAM), a glycolipid in the cell wall of tubercle bacilli.
- **Complement receptors** (C3b receptor) which bind mycobacteria opsonized by C3b.

Proliferation of mycobacteria within macrophages:

- Tubercle bacilli **proliferate freely** within the phagosome of the macrophage by **blocking fusion of the phagosome and lysosome**. It may result in **bacteremia** and seeding

of tubercle bacilli at many sites. This bacteremia may be either asymptomatic or may have a mild flu-like illness.

- **Genetic factor: NRAMP1 is a transmembrane protein** (a product of *NRAMP1* gene) **inhibits microbial growth**. In individuals with **polymorphisms in the NRAMP1** (natural resistance-associated macrophage protein 1) **gene, tuberculosis may progress due to the absence of an effective immune response**.

Cord factor is a virulent factor for *Mycobacterium tuberculosis*.

Mycobacterial glycolipid (lipoarabinomannan) blocks the fusion of phagosome with lysosome in the alveolar macrophage.

Initiation and Consequences of Cell-mediated Immunity

Cell-mediated immunity develops about 3 weeks after exposure.

Presentation of antigen to CD4⁺ T-cells:

Antigen-presenting cells (APCs which includes macrophages, dendritic cells) process the mycobacterial antigen and present it to naïve CD4⁺ T cells. Processed antigen reaches regional lymph nodes.

Differentiation of CD4⁺ T-cells into T-helper 1 (T_H1) cell:

It is due to the action of IL-12 secreted by macrophages.

Role of T_H1 cells:

Tuberculosis: T_H1 cells produce IFN-γ and plays a crucial role in the development of granulomatous lesion.

- **T_H1 cells** produce IFN-γ which has several functions.
 - **Activation of macrophages:** IFN-γ activates macrophages to contain the *M. tuberculosis* infection. These macrophages become bactericidal and secrete TNF → which promotes recruitment of more monocytes → **differentiation into epithelioid (epithelium-like) cells.**
 - **Stimulates formation of the phagolysosome** in infected macrophages → exposes the bacteria to acidic environment.
 - **Stimulates nitric oxide synthase** → to produce nitric oxide (NO) → NO + other oxidants → create reactive nitrogen intermediates → antibactericidal.
 - **Generation of reactive oxygen species** → antibacterial.
 - Mobilizes antimicrobial peptides (defensins) against the mycobacteria.
 - Stimulates autophagy (destroys damaged organelles and intracellular *M. tuberculosis*).
- T_H1 is also responsible for the **formation of granulomas and caseous necrosis.**

Granulomatous inflammation

- **Transformation of macrophages into epithelioid cells:** Activated macrophages are transformed into epithelioid cells. Some of these “epithelioid cells” may fuse to form giant cells.
- **Granuloma formation:** A microscopic aggregates of epithelioid cells, surrounded by a rim of lymphocytes, is referred as a granuloma and this pattern of inflammation, known as **granulomatous inflammation**. **Hypersensitivity reaction results in tissue destruction → caseation and cavitation.**

Epithelioid granuloma is caused by: Helper T-cells.

Primary Tuberculosis**Q. Write short note on primary tuberculosis.**

- **Initial infection** that occurs on first exposure to the organism in an **unsensitized** (previously unexposed) individual is known as primary tuberculosis.

Primary tuberculosis: Initial infection in an unsensitized individual.

- About 5% of newly infected people develop clinically significant disease.
- **Source** of the organism is always **exogenous**.

Sites of primary tuberculosis: Lung, intestine, tonsil, skin (very rare).

MORPHOLOGY**Sites of Primary Tuberculosis**

Lung, intestine, tonsil and skin (very rare).

Lung: It is the **commonest** site of primary tuberculosis.

- **Ghon lesion:** Following inhalation, tubercle bacilli gets deposited in the distal airspaces.
 - **Site of deposit:** Usually lower part of the upper lobe or upper part of the lower lobe near the pleural surface (subpleural).
 - **Ghon focus** (Fig. 16.18): About 2–4 weeks after the infection, a circumscribed **gray-white area** of about 1–1.5 cm develops in the lung known as the Ghon focus → the center of which undergoes caseous necrosis.

Q. Write short note on Ghon focus.

- **Regional lymphadenitis:** Tubercle bacilli (free or within macrophages) are carried along the lymphatics to the regional draining nodes → which often show caseous necrosis.
- **Ghon complex:** It is the combination of subpleural parenchymal lung lesion (**Ghon focus**) and **regional lymph node** involvement.

Q. Write short note on Ghon complex.

- **Fate of Ghon complex:**
 - **Healing:** In majority (about 95%), cell-mediated immunity controls the infection and primary tuberculosis **heals**. The **hallmark of healing is fibrosis** and Ghon complex undergoes progressive fibrosis, followed by radiologically detectable **calcification** (Ranke complex), and very rarely ossification.
 - **Spread:** During the first few weeks **lymphatic and hematogenous dissemination** occurs to other organs or parts of the body.

Primary tuberculosis: Ghon complex consists of Ghon lesion in the lung with regional lymphadenitis.

Ghon focus: This term is restricted only to primary tuberculosis of lung. In other organs, it is called as primary focus.

Other Sites of Primary Complex

- **Intestine:** Primary focus in the small intestine (usually ileal region) along with mesenteric lymphadenitis.
- **Tonsils:** Primary focus in the pharynx and tonsil with cervical lymph node enlargement.
- **Skin:** Primary focus in the skin along with regional lymph node involvement.

Microscopy (Fig. 16.19)

Granuloma in tuberculosis is called as tubercle. Tubercle may show central area of caseous necrosis (caseating granuloma) or may not show caseation (noncaseating tubercles). Individual tubercles are identified only by microscopic examination. When multiple granulomas coalesce → they may be grossly identified.

Caseating granuloma consists of:

- **Central area of caseous necrosis**
- **Surrounded by epithelioid cells** (modified macrophages), some of which may fuse to form multinucleate giant cells. The **giant cells may be Langhans type** (nuclei arranged in horse-shoe pattern) or **foreign body type** (nuclei in the center).
- The epithelioid cells are **surrounded by rim of lymphocytes**.
- These granulomas are usually enclosed within fibroblasts.

Tubercle: Granuloma in tuberculosis.

Caseating granuloma: Central area of caseous necrosis surrounded by epithelioid cells, lymphocytes and Langhans type of giant cells.

Epithelioid cells: Modified macrophages.

Immunocompromised patients usually do not form the characteristic granulomas.

Secondary Tuberculosis

(Synonyms: Post-primary tuberculosis, reactivation tuberculosis)

- **Tuberculosis developing in a previously sensitized individual is known as secondary tuberculosis.**
- It may follow shortly or many years after primary tuberculosis, usually when host resistance decreases.

- **Source of infection:**

- Most common source is **reactivation of a latent infection**.
- Rarely exogenous reinfection.

- Any location may be involved in secondary tuberculosis, but the lungs are by far the most common site.

Secondary tuberculosis: Infection in a previously sensitized individual. Source of infection may be reactivation of latent infection or reinfection.

MORPHOLOGY**Gross (Fig. 16.20)**

- **Site:** In the lungs, secondary tuberculosis usually involves the **apex of the upper lobes** of one or both lungs, **within 1–2 cm of the apical pleura**.
- **Appearance:** **Initially- small focus** (less than 2 cm in diameter) **of consolidation**, sharply circumscribed, firm and **gray-white to yellow** in color.
- **Regional lymph nodes involvement is less prominent** than in primary tuberculosis.

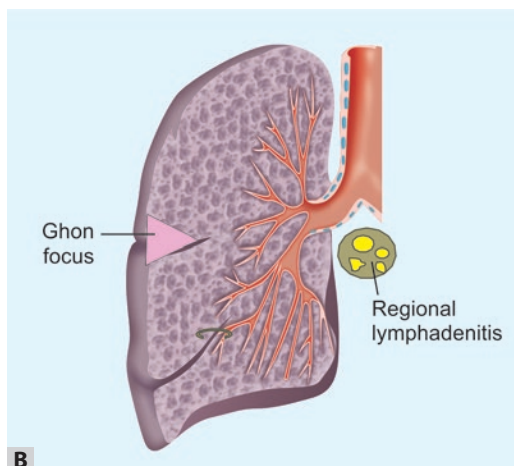
Microscopy

Active lesions show **caseating granulomas** and **acid-fast stain** often shows **tubercle bacilli**.

Natural history and various stages of tuberculosis are depicted in Figure 16.21.

Fate of Secondary Tuberculosis (Fig. 16.22)**Healing**

In immunocompetent individuals, localized, apical, focus may **heal with fibrosis and calcification**, rarely ossification.



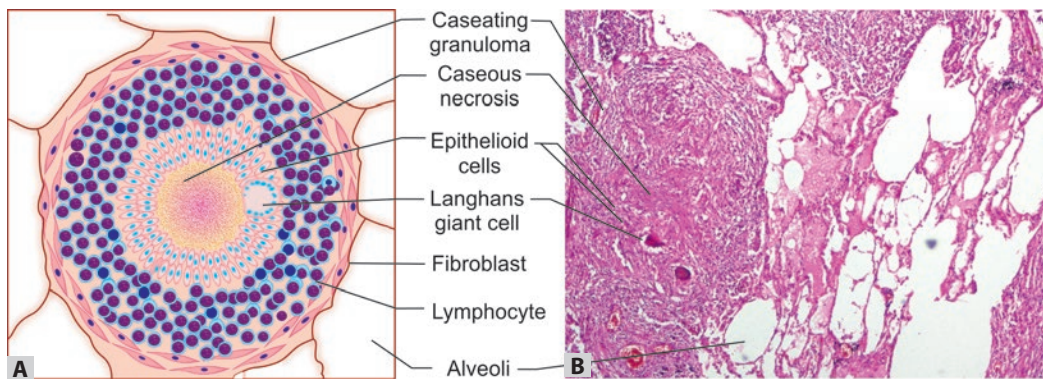
Figs 16.18A and B: (A) Gross appearance of primary pulmonary tuberculosis; (B) Diagrammatic. Ghon complex consists of gray-white parenchymal focus under the pleura and enlarged hilar lymph nodes with caseation

Acid-fast organisms

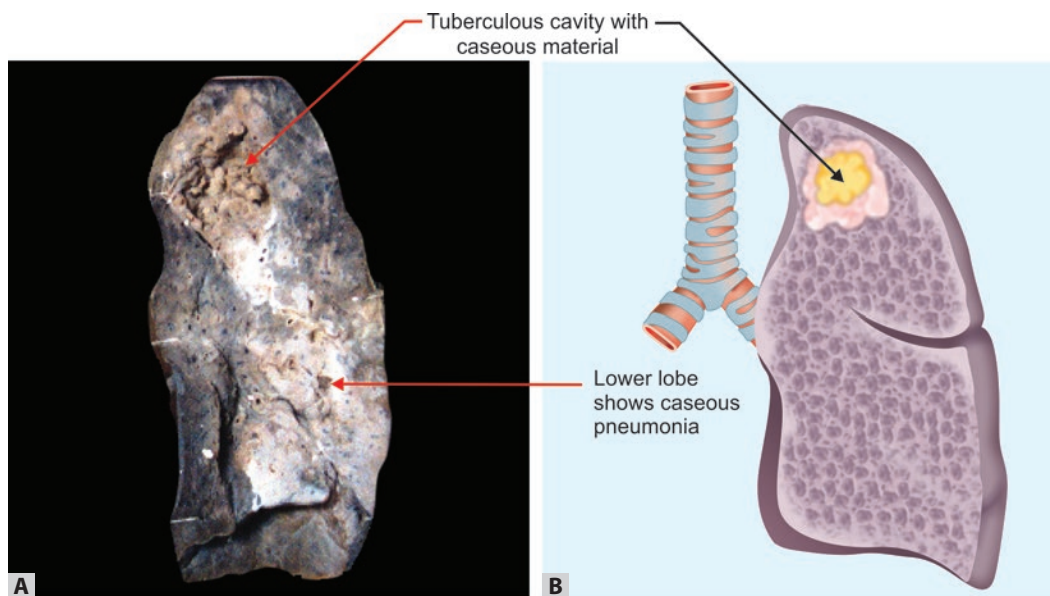
1. *Mycobacterium tuberculosis*
2. *M. leprae*
3. *M. smegmatis*
4. Atypical *Mycobacterium*
5. *Nocardia*
6. *Rhodococcus*
7. *Legionella micdadei*
8. Protozoa
 - Isospora
 - *Cryptosporidium parvum*

Acid-fast structures

- Head of human sperm
- Embryophora of *T. Saginata*
- Hooklets of *E. granulosus*
- Keratin



Figs 16.19A and B: Microscopic appearance of tuberculous lung. (A) Diagrammatic; (B) Photomicrograph. Central area of caseation surrounded by epithelioid and multinucleated giant cells



Figs 16.20A and B: Secondary pulmonary tuberculosis. (A) specimen; (B) diagrammatic. The lung shows gray-white areas of caseation with a cavity in the upper lobe

Assman focus:

Infraclavicular lesion of tuberculosis.

Simon focus: Healed calcified caseous nodule of tuberculosis at the apex of lung.

Rich focus: Tuberculous granuloma occurring on the cortex of the brain that ruptures into the subarachnoid space.

Simmond focus: Healed foci of TB in the liver.

Weigart (Wigard) focus: Healed foci of TB in the intima of blood vessels.

Progress

It may occur along several different pathways.

Progressive pulmonary tuberculosis

It occurs mainly in the elderly and immunosuppressed. Apical lesion may expand into surrounding lung and may erode into bronchi and vessels.

- **Erosion into bronchi:** It leads to release of the central area of caseous necrosis → resulting in a ragged, irregular **apical cavity** surrounded by fibrous tissue. This produces an **important source of infection**, because when the patient coughs, sputum contains bacteria.
- **Erosion of blood vessels:** May results in hemoptysis. With prompt treatment, it may undergo healing by fibrosis.

Spread of infection

If the treatment is inadequate or if host defenses are impaired, the infection may spread via: (i) Airways, (ii) lymphatics or (iii) Blood vessels.

1. **Local/direct spread:** Tuberculosis can directly spread to the surrounding tissue. In the lung, local spread to the pleura leads to serous **pleural effusions**, **tuberculous empyema**, or **obliterative fibrous pleuritis**.
2. **Spread through bronchi/airways:** It may produce tuberculous pneumonia.
 - **Intestinal tuberculosis:** In the past, intestinal tuberculosis resulted by the drinking of contaminated milk and with pasteurization of milk, now it is rare. Nowadays caused by the **swallowing of coughed-up infective material** in patients with open case of

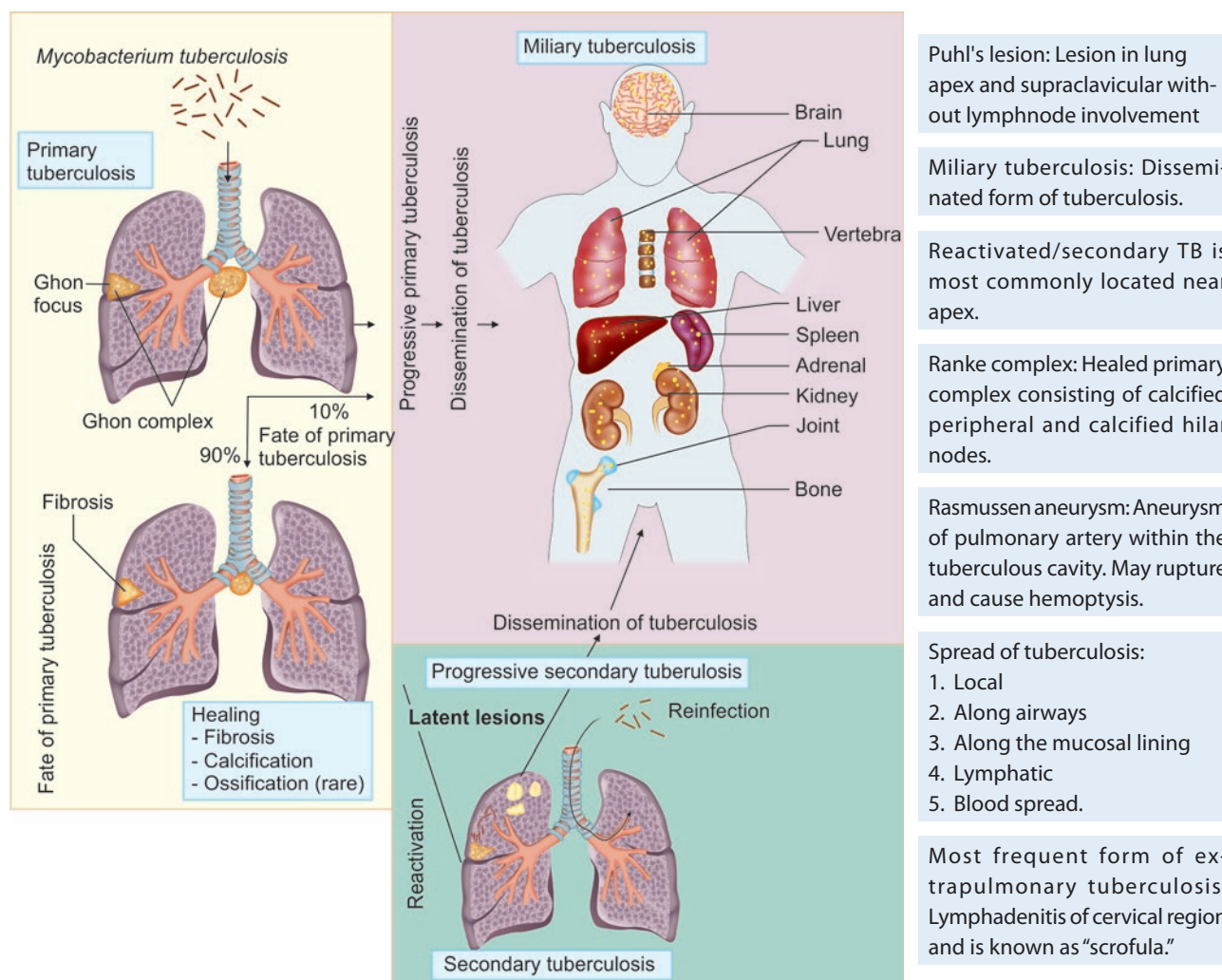


Fig. 16.21: Natural history and various stages of tuberculosis

advanced pulmonary tuberculosis. **Mainly develops in the ileum.**

3. **Spread along mucosal lining:** Spread through lymphatic channels or along the mucosal lining from mycobacteria present in the expectorated infectious material may lead to **endobronchial, endotracheal and laryngeal tuberculosis.**
4. **Lymphatic spread:** Spread through lymphatic channels mainly reach regional lymphnodes. It may also cause disseminated disease.
 - **Miliary pulmonary disease:** It is the disseminated form of tuberculosis. When the dissemination occurs only to the lungs, it is called miliary pulmonary disease.
 - **Mechanism of development:** Tubercle bacilli draining through lymphatics → enter the venous

blood → to the right venous side of the heart → through pulmonary artery → to the lung.

- **Lesions: Multiple, small, yellow, nodular** lesions in the lung parenchyma of both the lungs. Each lesion is either microscopic or small, visible (2-mm) foci of **yellow-white** consolidation → **resemble to millet seeds, hence named "miliary".**
- **Lymphadenitis:** It is most frequent presentation of extrapulmonary tuberculosis, and usually occurs in the cervical region ("scrofula").
 - HIV-negative individuals: Unifocal and localized.
 - HIV-positive individuals: Multifocal and systemic disease.
- 5. **Spread via blood vessels:**
 - **Systemic miliary tuberculosis** occurs when tubercle bacilli disseminate through the systemic arterial

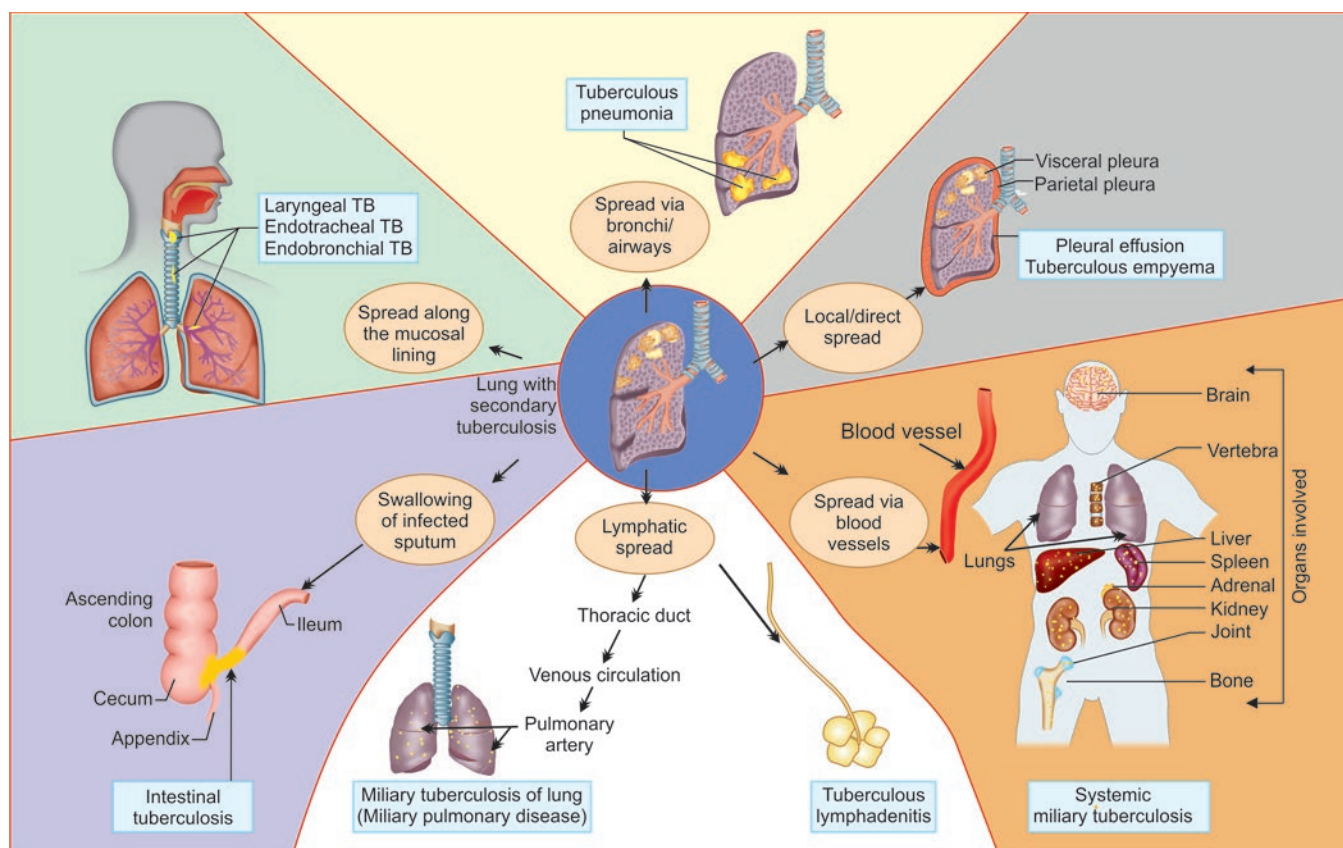


Fig. 16.22: Progress and complications of secondary tuberculosis of lung

system. Miliary tuberculosis most commonly involves liver, bone marrow, spleen, adrenals, meninges, kidneys, fallopian tubes, and epididymis. But no organ is exempt and can involve any organ.

- **Isolated-organ tuberculosis:** Dissemination of tubercle bacilli through blood may seed any organ or tissue → resulting in isolated organ tuberculosis. Commonly involved organs are as follows:
 - Meninges (tuberculous meningitis).
 - Kidneys (renal tuberculosis).
 - Adrenals.
 - Bones (osteomyelitis): When it involves the vertebrae, the disease is referred to as **Pott disease**. Paraspinal “cold” abscesses may track along tissue planes and present as an abdominal or pelvic mass.
 - Fallopian tubes (salpingitis).

Pott's disease: Tuberculosis of vertebrae.

6. Complications of secondary tuberculosis:

- **Hemoptysis** due to erosion into small pulmonary arteries adjacent to the tuberculous cavity.

- **Bronchopleural fistula:** It may develop when a tuberculous cavity in the subpleural region ruptures into the pleural space. It may lead to tuberculous empyema and pneumothorax.
- **Aspergilloma:** It is a fungal mass produced due to superinfection of a persistent open cavity with *Aspergillus*. It may fill the entire cavity and form a fungal ball.

Clinical Features

Tuberculosis of lung: Hemoptysis is the beginning of end or end of the beginning:

- William Boyd.
- Localized secondary tuberculosis may remain asymptomatic.
- **Systemic symptoms of pulmonary tuberculosis:** Probably due to cytokines released by activated macrophages (e.g., TNF and IL-1).
 - **Nonspecific:** Malaise, anorexia, weight loss, and fever.
 - **Low-grade fever:** It is **remittent** (appearing late each afternoon and then subsiding—commonly known as **evening rise of temperature**), and **night sweats**.

- **Sputum:** It is first mucoid and later purulent.
- **Hemoptysis:** It is present in 50% of cases of pulmonary tuberculosis.
- **Pleuritic pain:** Due to infection to the pleural surfaces.
- **Extrapulmonary manifestations** depend on the organ/system involved.

Patients with HIV infection have increased risk of tuberculosis.

Bronchial artery is the source of hemoptysis in tuberculosis

Lung: Granuloma with necrosis is seen in:

1. Tuberculosis
2. Histoplasmosis
3. Wegener's granulomatosis
4. Cryptococcosis.

Diagnosis: Pulmonary Disease

Q. Write short note on diagnosis of tuberculosis.

- Based on the **history, physical and radiographic** findings.
- Identification of **acid-fast tubercle bacilli in the sputum**.
- **Culture of the sputum:** Conventional cultures require up to 10 weeks. Culture is the gold standard.
- **PCR amplification** of *M. tuberculosis*: DNA is the rapid method of diagnosis. PCR can detect as few as 10 organisms in clinical specimens.

Prognosis: Generally good if infections are localized to the lungs. All stages of HIV infection are associated with an increased risk of tuberculosis.

Main difference between primary and secondary tuberculosis of lung are presented in Table 16.3.

SARCOIDOSIS

Definition: Sarcoidosis is a multisystem disease of unknown cause characterized by the presence of **noncaseating granulomas** in many tissues and organs.

- **Organs involved:** Sarcoidosis can affect every organ of the body. **It most commonly** affects the **lung** and the **lymph nodes** in the **mediastinum and hilar regions**. Other organs commonly affected are the liver, skin, and eye.
- **Age and gender:** Most patients are young (from 20 to 40 years of age) and more common in women than in men.

Etiology

Exact cause of sarcoidosis is **not known**. Factors that play a role in its pathogenesis are as follows: (i) Environmental, (ii) genetic, and (iii) immunological.

- **Environmental factors:** Environmental agents (infectious and noninfectious) have been suggested as possible causes. Infectious inciting agent includes: *Mycobacteria* (both *Mycobacterium tuberculosis* and nontuberculous mycobacteria), *Propionibacterium acnes*, *Borrelia burgdorferi*, viruses, fungi, spirochetes and *Rickettsia*.
- **Genetic factors:** Certain specific HLA genotypes (e.g. HLA-A1 and HLA-B8) are associated with an increased risk for developing sarcoidosis.
- **Immunological factors:** They are important in pathogenesis. The most likely etiology is an environmental agent (infectious or noninfectious) that triggers cell-mediated immune response to an unidentified antigen in a genetically susceptible host.

TABLE 16.3: Main difference between primary and secondary tuberculosis of lung

Characteristics	Primary TB	Secondary TB
Type of infection	Initial infection in an unsensitized individual	Infection in a previously sensitized individual
Source of infection	Exogenous	Usually endogenous activation of latent infection
Site of lung involved	Lower part of upper lobe or upper part of lower lobe and subpleural	Apex and posterior segments of the upper lobe and superior segments of lower lobe
Regional lymph node involvement	Significant and prominent	Not prominent
Sputum for AFB	Rarely positive	Commonly positive
Cavity formation	Rare	Common
Healing	Mainly by calcification	By fibrosis
Extrapulmonary complications	Very common	Lesion usually localized to lung

Clinical features of tuberculosis:

1. Drenching night sweats
2. Fever
3. Weight loss.

Most common cause of drug-resistant tuberculosis is previous anti-tuberculous treatment (ATT).

Pathogenesis

The granuloma is the pathologic hallmark of sarcoidosis. A characteristic feature of sarcoidosis is the local accumulation of inflammatory cells. The sarcoid granulomas develop as a **cell-mediated response to an unidentified antigen**. The sequences of events are as follows:

- An unknown antigen is taken up by antigen-presenting cells.
- APC present the unknown antigen to the CD4⁺ helper T cells. This results in antigen-driven proliferation of CD4⁺ helper T cells and accumulation of activated monocytes.
- CD4⁺ helper T cell release several cytokines, such as:
 - Interleukin (IL)-2 results in T-cell expansion.
 - Interferon- γ (IFN- γ) causes macrophage activation.
- Local increase of several cytokines (IL-8, TNF, macrophage inflammatory protein 1 α) favors recruitment of additional T cells and monocytes and contributes to the formation of granulomas.

Sarcoidosis: Raised interleukin (IL)-2 results in T-cell expansion.

Systemic Immunological Abnormalities

Patients with sarcoidosis may also show:

- **Anergy to common skin test antigens**, such as tuberculosis purified protein derivative (PPD) or as *Candida*.
- **Polyclonal hypergammaglobulinemia** (another manifestation of helper T-cell dysregulation).

Sarcoidosis: Anergy to skin test antigens, such as tuberculosis purified protein derivative (PPD) or as *Candida*.

MORPHOLOGY (FIG. 16.23)

Noncaseating Granulomas

- The involved tissues show the classical well-formed **noncaseating granulomas**.
- They are composed of an **aggregate of epithelioid cells and lymphocytes** (mainly CD4⁺ T-cell). Epithelioid cells are modified macrophages and characteristically have abundant eosinophilic cytoplasm and vesicular nuclei.
- Langhans or foreign body-type giant cells may also be seen.
- **Central necrosis is not seen.**

Other findings: Two other microscopic findings of sarcoidosis include:

- **Schaumann bodies:** These are laminated concretions of calcium and proteins.
- **Asteroid bodies:** These are stellate inclusions within giant cells in about 60% of the granulomas.

They are not pathognomonic because they can be seen in other conditions.

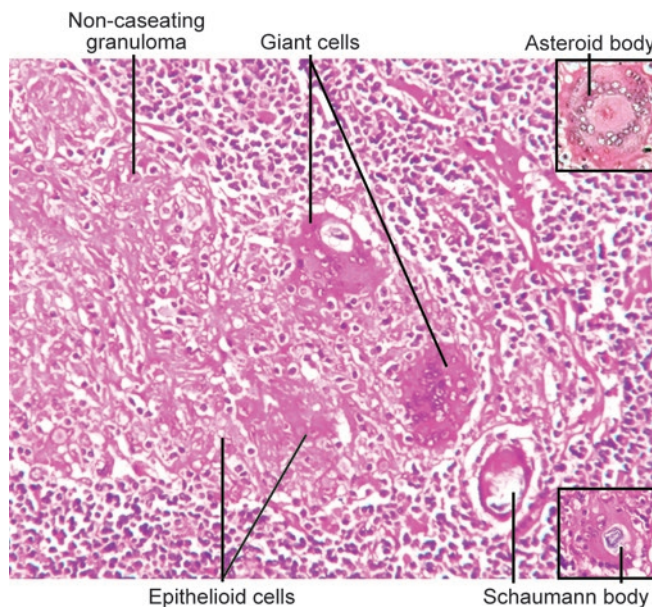


Fig. 16.23: Microscopic appearance of sarcoidosis showing non-caseating granuloma composed of epithelioid cells surrounded by lymphocytes and giant cells. Inset shows giant cell with asteroid body (upper right) and Schaumann body (lower right)

Sarcoidosis: Noncaseating granuloma.

Sarcoidosis: Schaumann bodies-laminated concretions of calcium and proteins.

Sarcoidosis: Asteroid bodies

- Stellate (star shaped) inclusions within giant cells.

Fate of Granuloma

- The granulomas may resolve completely.
- As the lesion become chronic, proliferation of collagen forms concentric laminated layer giving them an onion-skin appearance and later may form nodular hyaline scars.

Sarcoidosis: Polyclonal hypergammaglobulinemia.

Diagnosis

- **Involvement of two or more organs is necessary.**
- **Histologic diagnosis** is made **after exclusion** of organisms that are known to be associated with granuloma formation. Noncaseating (hard) granuloma is not specific for sarcoidosis, and may be seen in (i) mycobacterial infections, (ii) fungal infections, (iii) malignancy, and (iv) environmental agents (e.g. beryllium).

Lung

The **lungs** are most commonly involved.

MORPHOLOGY

- **Gross:** No gross changes in early stage. In advanced cases the coalescence of granulomas produces **small palpable or visual nodules** measuring 1–2 cm.
- **Microscopy:** The **granulomas** are found in the **pulmonary interstitium** along the **lymphatics, around bronchi and blood vessels**, although alveolar lesions are also seen. The lesions in the lung may heal with fibrosis and hyalinization are often found.

Lymph Nodes

They are involved in almost all cases, particularly the hilar and mediastinal nodes. Lymphnodes are enlarged, discrete, and sometimes calcified.

Clinical Features

- Variable clinical manifestations.
- Insidious onset of respiratory symptoms (shortness of breath, cough, chest pain, hemoptysis) or of constitutional signs and symptoms (fever, fatigue, weight loss, anorexia, night sweats).
- May be detected on **routine chest films as bilateral hilar lymphadenopathy** or may present with peripheral lymphadenopathy, skin lesions, eye involvement, splenomegaly, or hepatomegaly.

Sarcoidosis: Diagnosis is by exclusion of other granulomatous diseases.

Kveim test: For diagnosis of sarcoidosis.

Sarcoidosis:

1. Increased ACE
2. Hypercalcemia due to hypervitaminosis D.

Prognosis

Variable and is good for the majority of patients.

Recovery with resolution of the disease: Occurs either spontaneous or induced by steroid therapy in about 65–70% of patients.

Chronic or progressive course: It may lead to permanent loss of some lung function or some permanent visual impairment in about 25% of patients.

Death: Less than 3% of patients may die due to cardiac or pulmonary involvement.

ACUTE LUNG INJURY AND ACUTE RESPIRATORY DISTRESS SYNDROME (DIFFUSE ALVEOLAR DAMAGE)

Q. Write short essay/note on acute lung injury/ARDS/Diffuse alveolar damage.

- **Acute lung injury (ALI)** is also termed as noncardiogenic pulmonary edema characterized by: **1) abrupt onset of significant hypoxemia and 2) bilateral pulmonary infiltrates in the absence of cardiac failure.**
- **Acute respiratory distress syndrome (ARDS)** is a severe form of ALI.
- ARDS as well as ALI has inflammation associated increase in pulmonary vascular permeability, edema and death of epithelial cells. Histologically they show **diffuse alveolar damage (DAD).**

Etiology

ALI is triggered by diverse conditions (Box 16.4)

BOX 16.4: Etiological factors of acute respiratory distress syndrome

- **Infection**
 - Sepsis: E.g. Gram-negative septicemia
 - Lung infections (viral, *Mycoplasma*, and *Pneumocystis* pneumonia, miliary tuberculosis)
 - Aspiration of gastric contents
- **Physical injury**
 - Mechanical trauma
 - Fat embolism
 - Lung contusions
 - Burns
 - Near-drowning
 - Ionizing radiation
- **Inhalation of toxic gases and chemicals**
- **Chemical injury**
 - Overdose of heroin, methadone, barbiturate
 - Acetylsalicylic acid
 - Cytotoxic drugs
- **Hematological conditions**
 - Transfusion associated lung injury (TRALI)
 - Disseminated intravascular coagulation (DIC)
- **Pancreatitis**
- **Uremia**
- **Hypersensitivity reactions**

Pathogenesis

ALI/ARDS starts when there is injury to pneumocytes and pulmonary endothelium and creates a viscous cycle of inflammation and pulmonary damage.

- **Endothelial cell injury/activation:** It is an important early event.

- **Adhesion and extravasation of neutrophils:** Neutrophils adhere to the activated endothelium and migrate into the interstitium and the alveoli. In the alveoli, neutrophils release inflammatory mediators, proteases, reactive oxygen species, and cytokines. This further recruits leukocytes, causing further damage to endothelium. This cycle of inflammation and endothelial damage is the basic mechanism of ALI/ARDS.
- **Formation of hyaline membrane:** Endothelial activation and injury causes leakage of fluid from pulmonary capillaries into the interstitial tissue of lung. Loss of type I pneumocytes permits fluid to enter the alveolar spaces. Injury to type II alveolar pneumocytes produces surfactant abnormalities which compromises the alveolar gas exchange. The protein-rich (derived from leaked plasma) edema fluid and debris from dead alveolar epithelial cells form hyaline membranes which is characteristic of ALI/ARDS.
- **Resolution of injury:** If the patient survives the acute phase, macrophages remove intraalveolar debris and release fibrogenic cytokines [e.g. transforming growth factor β (TGF- β) and platelet derived growth factor (PDGF)]. These cytokines stimulate fibroblast growth and collagen deposition in the alveolar walls resulting in fibrosis of alveolar walls. Bronchiolar stem cells replace pneumocytes and uninjured capillary endothelium replace the endothelial cells.

MORPHOLOGY

Gross

Lungs are heavy, firm, red and boggy.

Microscopy

- **Exudative stage**
 - Congestion, interstitial and intra-alveolar edema, accumulation of inflammatory cells, fibrin deposition, and **diffuse alveolar damage**. Interstitial and intra-alveolar edema is prominent by the first day.
 - **Hyaline membranes:** The alveolar walls become lined with waxy hyaline membranes which appear by the second day and are the most conspicuous morphologic feature. Alveolar hyaline membranes are eosinophilic and glassy. They consist of precipitated fibrin-rich plasma proteins in the edema fluid mixed with the cytoplasmic, nuclear and lipid debris from sloughed necrotic epithelial cells.
- **Organizing stage:** In this stage, type II pneumocytes proliferate, and granulation tissue grows in the alveolar walls and into the alveolar spaces. In majority, the granulation tissue resolves with only minimal functional impairment.

Clinical Course

Patients who develop ALI are usually admitted to the hospital for their predisposing conditions. Presenting features include severe dyspnea and tachypnea, increasing

cyanosis and hypoxemia, respiratory failure, and the appearance of diffuse bilateral infiltrates on chest X-ray.

ATELECTASIS (COLLAPSE)

Q. Write short note on atelectasis.

Atelectasis is the term used for either incomplete expansion of the lungs (neonatal atelectasis) or to the **collapse of previously expanded/ inflated lung** (acquired atelectasis). It produces relatively airless areas in the lung parenchyma.

Main Types of Acquired Atelectasis

- **Resorption atelectasis:** Develops due to complete obstruction of an airway. Causes include obstruction due to excessive secretions (e.g., mucus plugs) or exudates within smaller bronchi (e.g. bronchial asthma, chronic bronchitis, bronchiectasis, and postoperative states), aspiration of foreign bodies, and fragments of bronchial tumors (rare). Air in the dependent alveoli distal to obstruction gets resorbed and causes collapse of alveoli. There is reduced lung volume which shifts the mediastinum toward the atelectatic lung.
- **Compression atelectasis:** Develops due to **compression of lung from external aspect**. Causes include **accumulation of significant volumes of fluid** (transudate, exudate or blood) or **air** (pneumothorax) **in the pleural cavity** and compression by tumor. In this type of atelectasis, the mediastinum is shifted away from the affected lung.
- **Contraction atelectasis** develops when **focal or generalized pulmonary or pleural fibrosis prevents full expansion of the lung**.

Significance

- Significant atelectasis **reduces oxygenation and predisposes to infection**.
- In long-standing atelectasis, the collapsed area of lung becomes fibrotic and bronchi dilate, in part owing to infection distal to the obstruction—**bronchiectasis**.
- It is usually reversible disorder except contraction atelectasis.

PNEUMOCONIOSES

Q. Define and classify pneumoconiosis.

Definition: Pneumoconioses are defined as **lung diseases produced by organic as well as inorganic particulates and chemical fumes and vapors**.

Originally, the term pneumoconiosis (or dust diseases of the lung) was used for non-neoplastic lung reaction to inhalation of mineral dusts. Few disease caused by mineral dusts is shown in Table. 16.4.

TABLE 16.4: Most common lung diseases caused by mineral dusts

Causative agent	Disease	Occupation/exposure
Coal dust	Simple coal worker's pneumoconiosis (macules and nodules)	Coal mining
	Complicated coal worker's pneumoconiosis: Progressive massive fibrosis (PMF)	
Silica	Silicosis	Sandblasting, stone cuttings
Asbestos	Asbestosis	Mining, fabrication, and installation and removal of insulation
	Pleural plaques	
	Mesothelioma	
	Carcinoma of the lung, larynx, stomach, colon	

Pneumoconioses: Lung diseases produced by organic as well as inorganic particulates and chemical fumes and vapors.

Pathogenesis

Only a small percentage of exposed people develop pneumoconioses, and indicates a genetic predisposition. The development of pneumoconiosis depends on:

- Amount of dust retained in the lung and airways:** It depends on the concentration of dust in air, the duration of exposure, and the efficiency of host clearance mechanisms.
- Size, shape, and floating capacity of the particles:** Most dangerous particles range in size from 1 to 5 μm in diameter \rightarrow may reach the terminal small airways and air sacs and settle in their linings.
- Solubility and physiochemical reactivity of the particles:** It influenced by their size.
 - **Smaller particles** which are soluble in pulmonary fluids can reach toxic levels rapidly and **produce acute lung injury**.
 - **Larger particles** which are not soluble persist within the lung parenchyma for years \rightarrow produce pneumoconioses (e.g. silicosis).

- Additional effects of other irritants:** e.g. accompanying **tobacco smoking** \rightarrow **worsens the effects of all inhaled mineral dusts**.

Most dangerous particles causing pneumoconiosis: 1–5 μm .

Pneumoconiosis: Particle less than 0.5 μm reach the alveoli.

Coal Workers' Pneumoconiosis

Coal workers pneumoconiosis: Due to inhalation of carbon (anthracotic) pigment.

Definition: Coal workers' pneumoconiosis (CWP) is the **parenchymal lung disease caused by inhalation of carbon particles** (coal mine dust).

Pathogenesis

The pathogenesis of CWP is not completely known. In most cases, carbon dust itself is the major culprit. However, contaminating silica in the coal dust can also contribute to the development of coal workers' pneumoconiosis.

Dust cells: Alveolar macrophages with anthracotic (carbon) pigment.

MORPHOLOGY

Group of lung diseases caused by coal mine dust is commonly referred to as **black lung**. Lung lesions due to progressive accumulation of carbon particles can be divided into three stages.

- Pulmonary asymptomatic anthracosis**
 - It is a harmless coal-induced lesion in lungs of coal miners and also seen in persons residing in urban areas and tobacco smokers.
 - Inhaled carbon pigment is engulfed by macrophages present in the alveoli or interstitium of lung \rightarrow accumulate in the connective tissue \rightarrow also carried by the lymphatics to the regional lymph nodes.
- Simple CWP with little/no pulmonary dysfunction.**
 - **Gross:**
 - **Coal macule:** Characteristic **nonpalpable** lesion that measure 1–2 mm in diameter.
 - **Coal nodule:** Larger, but less than 2 cm, palpable, round or irregular and firm lesions.
 - Both are mainly seen in the **upper lobes and upper zones of the lower lobes**, adjacent to respiratory bronchioles. As the lesion progresses, dilation of adjacent alveoli may produce **centrilobular emphysema**.
 - **Microscopy:**
 - **Coal macule:** It consists of **focal collections of many carbon-laden macrophages** surrounding respiratory bronchioles.
 - **Coal nodule:** It consist of carbon-laden macrophages and **small amounts of collagen and reticulin fibers**.

3. **Complicated CWP (progressive massive fibrosis—PMF)**

Develops in individuals with simple CWP and may have impaired respiratory function.

- **Gross** (Fig. 16.24): Lung shows **dark, multiple, black scars larger than 2 cm** (sometimes up to 10 cm in greatest diameter).
- **Microscopy**: Shows **carbon pigment**, carbon-laden macrophages, **dense collagen** and **disrupted lung's architecture**.

CWP: Not associated with risk of cancer or tuberculosis.

Clinical Course

CWP is usually associated with minimal loss of lung function. However, in less than 10%, PMF develops → leads to pulmonary dysfunction, pulmonary hypertension, and cor pulmonale.

Silicosis

Q. Write a note on silicosis.

Definition: Silicosis is a **parenchymal lung disease** associated with **inhalation of crystalline silicon dioxide** (silica).

- Most prevalent slowly progressive pneumoconiosis and usually present many years after exposure.
- Crystalline silicon dioxide is abundant and found in the earth's crust and is used in a variety of industrial applications.
- **Susceptible individuals:** Sandblasters, stone cutting, polishing and sharpening of metals, ceramic manufac-

turing, foundry work, tunneling through rock with high quartz content and the cleaning of boilers.

Pathogenesis

Forms of silica: It occurs in both **crystalline** and **amorphous** forms. The crystalline forms include quartz, cristobalite, and tridymite are much more toxic and fibrogenic.

Steps:

- **Inhalation:** Of all the crystalline forms, **quartz is most common cause of silicosis**. These particles reach lung by inhalation.
- **Interaction with epithelial cells and macrophages:** Silica is cytotoxic to alveolar macrophages. After inhalation, the particles reach the alveoli → **interact with alveolar epithelial cells** → ingested by alveolar macrophages → activates macrophages to **release of mediators** (IL-1, TNF, fibronectin, lipid mediators, oxygen-derived free radicals, and fibrogenic cytokines) → **stimulate fibroblast proliferation and collagen formation**.

Silicosis:

- Nodular fibrosing disease due to inhalation of silica
- Most common chronic occupational disease in the world.

Silicosis: Crystalline forms of silica are most toxic and fibrogenic.

MORPHOLOGY

Silicosis: Involves upper lobes of the lung.

Chronic silicosis occurs in **two forms**, namely, **simple** form and **progressive massive fibrosis**. Simple silicosis is not associated with significant respiratory dysfunction.

Gross

- **Simple nodular silicosis** (Fig. 16.25A): Represents early stage.
 - Shows **tiny, barely palpable, discrete** pale to blackened (if coal dust is also present) **silicotic nodules** (usually 2–4 mm) in the upper zones.
 - **Hilar lymph nodes** may become enlarged. The periphery of the lymph node may show thin rim of calcification and are seen radiographically as **eggshell calcification** (i.e. calcium deposition surrounding a zone lacking calcification).
- **Progressive massive fibrosis** (Fig. 16.25B): As the disease progresses, expansion and coalescence of lesions may produce progressive massive fibrosis.
 - Progressive massive fibrosis is defined radiologically as nodular masses of more than 2 cm diameter in a background of simple silicosis.
 - The smaller silicotic nodules coalesce to form **large lesions**, which range from 5 to 10 cm in size. These lesions are **hard** and some of them may undergo central softening and cavitation. These changes may be due to superimposed tuberculosis or to ischemia.
 - Fibrotic lesions may also develop in the hilar lymph nodes and pleura.

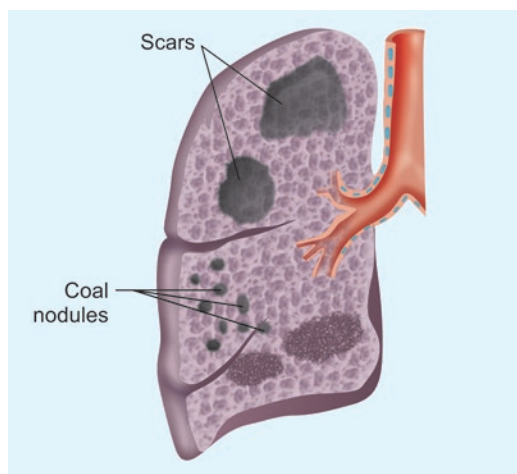
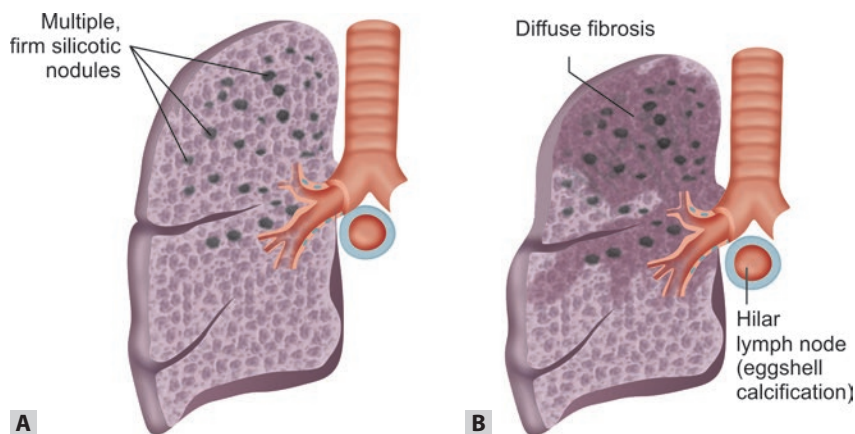


Fig. 16.24: Complicated coal worker's pneumoconiosis showing two large black scars in the upper part of lung

Caplan syndrome: Coexistence of rheumatoid arthritis with pneumoconiosis.



Figs 16.25A and B: Gross changes in silicosis. (A) Simple nodular silicosis; (B) Progressive massive fibrosis shows scarring at the upper lobe

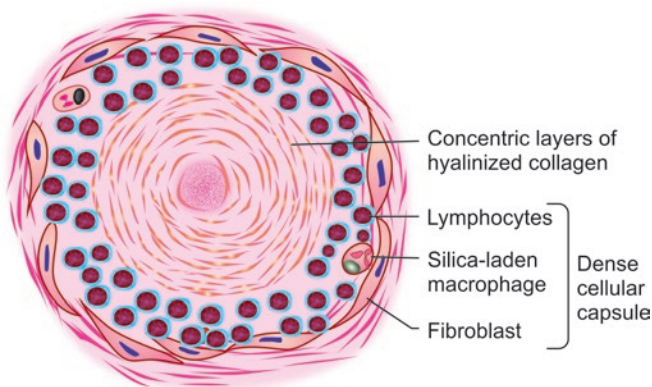


Fig. 16.26: Silicotic nodule is composed of concentric whorls of dense, sparsely cellular collagen. The edge of the nodule shows silica-laden macrophages and lymphocytes

Silicosis: Opacities in the lung-contain collagen and quartz.

Microscopy

- **Silicotic nodule** (Fig. 16.26):
 - It has a characteristic **whorled appearance**.
 - **Center:** It consists of **concentric layers of hyalinized acellular collagen** surrounded by a **dense cellular capsule** of more condensed collagen.
 - **Periphery:** Shows **aggregates of mononuclear cells**, mostly **macrophages, lymphocytes and fibroblasts**.
- **Polarized light microscopy** may show **birefringent silica particles** in the center of silicotic nodule.

Silicosis: Polarizing microcopy-birefringent silica particles.

Clinical Course

- In simple nodular silicosis, lung functions are either normal or only moderately affected.

Lung in silicosis:

- Hard collagenous scars
- Egg-shell calcification.

Silicosis: Chest X-ray shows eggshell calcification of hilar lymph nodes.

Silicosis: Extensive nodular pulmonary fibrosis.

- Chest radiographs typically show a fine nodularity in the upper zones of the lung.
- The patients with progressive massive fibrosis develop shortness of breath.
- The patients have **increased susceptibility to lung infections, such as *Mycobacterium tuberculosis*, atypical mycobacteria and fungi.**
- The crystalline silica is also carcinogenic.

Silica: Increased risk of cancer of lung and tuberculosis.

Asbestosis and Asbestos—Related Diseases

Q. Write a note on asbestosis.

Definition: Asbestosis is defined as interstitial fibrosis of the lung caused by exposure to **asbestos dust**. It does not include asbestos-induced pleural diseases and carcinoma of lung that are found in asbestos-exposed workers.

Asbestosis: Interstitial fibrosis of the lung caused by exposure to asbestos dust.

Asbestos Use and Exposure

Q. Write a note on asbestosis-related diseases.

- Asbestos is a family of crystalline hydrated silicates that form fibers.
- They have unique physical-chemical properties that make them effective for insulation, reinforcing materials, and friction products.
- Asbestos is used for fireproofing, in heat and sound insulations and for strengthening plastics and cement.

Asbestos-related diseases

1. Asbestosis
2. Localized fibrous plaques
3. Pleural effusions
4. Carcinoma of lung
5. Malignant mesotheliomas
6. Laryngeal carcinomas.

Asbestos Types

Asbestos is a generic term used for naturally occurring fibrous silicates. Two major forms are: **Serpentine and amphibole**.

1. **Serpentine form:**

- **Chrysotile** (white asbestos) is the **most important** serpentine form **asbestos used in industry**.
- Chrysotiles **consists of long, curly, flexible structure** → are likely to become impacted in the upper respiratory passages and removed by the mucociliary action.
- They are less readily carried to the lungs and even if trapped in the lungs, they fragment into short particles and easily ingested by macrophages.
- They are also **more soluble than amphiboles**.

2. **Amphibole form:**

- The main amphibole forms are **crocidolite (blue asbestos) and amosite (brown asbestos)**.
- Amphibole form **consists of straight, rigid, brittle fibers** that may align themselves in the airstream and remain stable in the lung.
- They neither fragment nor are soluble and thus reach deeper into the lungs, where they can penetrate epithelial cells and reach the interstitium.
- Though they are not commonly used, they are **more pathogenic than chrysotiles** particularly with respect to induction of malignant pleural tumors (mesotheliomas).

Pathogenesis**Fibrogenic Effect**

Inhaled asbestos fibers reach the air spaces → macrophages (alveolar and interstitial) attempt to ingest and clear the fibers → **activates the macrophages** → **release chemotactic factors and fibrogenic mediators**. Both **amphibole and serpentine forms are fibrogenic**. The initial injury occurs at bifurcations of small airways and ducts, where the asbestos fibers land and penetrate. Chronic exposure and deposition of asbestos fibers lead to persistent release of fibrogenic mediators. **The end result is generalized interstitial pulmonary inflammation and interstitial fibrosis.**

Oncogenic Effect

Asbestos can act both as a tumor **initiator and as promoter**.

- **Reactive free radicals:** These are produced by asbestos fibers → oncogenic.
- **Potentially toxic chemicals:** Inhaled toxic chemical are adsorbed onto the asbestos fibers → oncogenic. For example, the adsorption of carcinogens in **tobacco smoke** onto asbestos fibers → **increases the risk of lung carcinoma in asbestos workers**.

Asbestos:

- Fibrogenic
- Oncogenic.

Asbestos: Exposure to increasing doses are associated with a higher incidence of all asbestos-related diseases except mesothelioma, which is only associated with amphibole exposure.

MORPHOLOGY**Gross (Fig. 16.27A):**

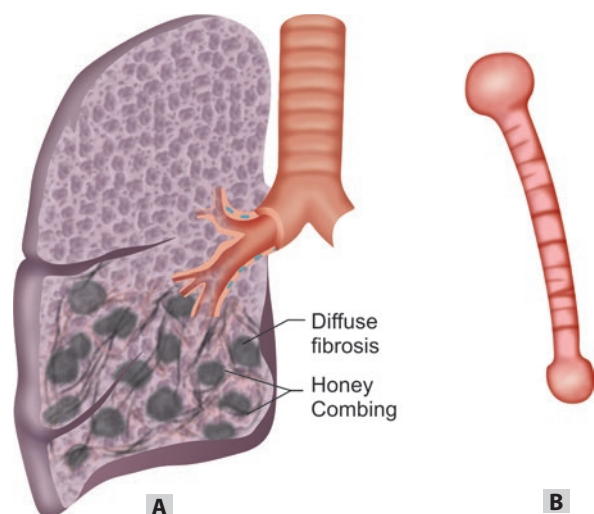
- **Sites:** Lesions **begin** in the **lower lobes and subpleura**.
- **Early stages:** Interstitial fibrosis may not be apparent on gross examination. Begins as interstitial **fibrosis around respiratory bronchioles and alveolar ducts** and extends to involve adjacent alveolar sacs and alveoli. The lesions are **termed mineral dust-induced small airways disease**.
- **Later stages:** The **interstitial fibrosis becomes more extensive and may involve the entire lung**. The fibrosis may **destroy the normal architecture of the lung** to produce **dilated airspaces (cystic spaces) surrounded by thick fibrous walls** → produces **honeycombed** appearance to the involved regions. The lungs become small.

Asbestosis: Common site is

- Lower lobe of lung
- Subpleura.

Microscopy

- **Asbestos bodies** (Fig. 16.27B):
 - The asbestos fibers after reaching the finer bronchioles and alveoli may form → asbestos bodies.
 - They **consist of translucent center of asbestos fibers coated with a film of proteins rich in iron**.
 - Asbestos bodies appear as **golden brown, fusiform or beaded rods**.
 - The coating is thickest at the ends of the fibers, giving a **dumb-bell appearance**.
 - They form when macrophages attempt to phagocytose asbestos fibers; the iron is probably derived from phagocyte ferritin.
- **Ferruginous bodies:** Other inorganic particulates/fibers may also become coated with similar iron-protein complexes → called as **ferruginous bodies**.
- **Fibrosis of the lung:** Lung shows **diffuse pulmonary interstitial fibrosis**, with multiple **asbestos bodies**.



Figs 16.27A and B: (A) Asbestosis of lung showing honeycomb appearance in the lower lobe; (B) Asbestos body is a ferruginous body, golden brown, beaded and having dumb-bell shape

Asbestos body: Asbestos fibers coated by iron.

Asbestosis: Asbestos bodies, Ferruginous bodies.

Asbestos body: Hallmark of asbestosis.

Asbestos body: Iron containing proteinaceous material coating asbestos fiber.

Ferruginous body: Iron protein complex coating other inorganic particles, such as talc, mica and glass in the lung (not asbestos fiber).

Complications: Pulmonary hypertension and cor pulmonale.

Pleural Plaques

Benign pleural plaques: Most common lesions in asbestosis

They are most common manifestation of asbestos exposure.

- **Gross:** Pleural plaques appear as **pearly white; well-circumscribed plaques** (may be over 10 cm in diameter). Usually **bilateral** and most frequent on the **anterior and posterolateral aspects of the parietal pleura** and **over the domes of the diaphragm**.
- **Microscopy:** It consists of **acellular, dense, hyalinized fibrous tissue**, with many **slit-like spaces** arranged in a parallel fashion (**basket-weave pattern**). May show calcification.

- They **neither show asbestos bodies nor do they progress to mesotheliomas**.

Asbestos-related disease: Lung cancer is the most common.

Asbestosis: Mesothelioma arises from the pleura.

Asbestosis: No increased risk of tuberculosis.

Lung Cancer and Mesothelioma

The workers exposed to asbestos may develop lung carcinomas and mesotheliomas (pleural and peritoneal). Concomitant cigarette smoking markedly increases the risk of lung carcinoma but not that of mesothelioma.

Clinical Features

- Initially patients present with **dyspnea on exertion**, but **later** it is present **even at rest**.
- Usually it is accompanied by productive cough.
- Chest X-rays show irregular linear densities, particularly in both lower lobes.
- As **the disease progresses**, a **honeycomb pattern** develops → may progress to respiratory failure, cor pulmonale, and death.
- Pleural plaques are usually asymptomatic and are visualized on radiographs as circumscribed densities.

Bagassosis is lung disease caused by: Sugarcane.

Byssinosis: Interstitial lung disease caused by organic dust and is associated with hypersensitive pneumonitis.

LUNG CARCINOMAS

- Carcinoma of the lung is the **most common cause of cancer death**. This is mainly due to the carcinogenic effects of **cigarette smoke**.
- In the past, the term bronchogenic carcinoma was used for primary lung cancer, to indicate the origin from the bronchi. Now it is known that about 5% of primary lung cancers do not arise from bronchus. Hence, the term lung cancer is used.
- **Age and gender:** Mostly found between **50 and 80 years** of age. More common in males, but there is a recent increase in females due to increased smoking among females.

Etiology and Pathogenesis

Q. Discuss etiopathogenesis of carcinoma of lung.

Tobacco Smoking

Lung cancer: Cigarette smoking is the most common cause.

Lung cancer: Nonsmokers usually develop adenocarcinoma.

Major carcinogens in tobacco smoke (Table 16.5): These include both initiators and promoters (such as phenol derivatives).

- **Polycyclic aromatic hydrocarbons:** Benzo[*a*]pyrene, dibenzanthracene.
- **Radioactive elements and other contaminants:** Arsenic, nickel, cadmium, molds, and vinyl chloride.

There is strong (i) statistical, (ii) epidemiological, (iii) clinical and (iv) experimental evidence that tobacco smoking is the most important cause of cancer of lung. Smoking also multiplies the risk of other carcinogenic influences, such as asbestos and uranium. The **strongest association** of smoking is with **squamous cell and small cell carcinoma**.

- **Statistical evidence:** About **90% of lung carcinomas occur in smokers** and it depends on:
 - Amount of daily smoking.
 - Tendency to inhale.
 - Duration of the smoking habit.

The **risk increases with number of cigarettes smoked** and is **proportional to the duration of smoking**. Compared to nonsmokers, the smokers have a 10 times and heavy smokers (more than 40 cigarettes per day for several years) 60 times more risk of lung cancer. Females are more susceptible to tobacco carcinogens than males. Cessation of smoking for 10 years reduces risk but not to the level in nonsmokers. However, only 11% of heavy smokers develop lung cancer, which indicates that genetic factors are involved. **Pipe and cigar smokers have lower incidence than cigarette smokers**.

TABLE 16.5: Selected tobacco smoke constituents and its effect

Constituent in tobacco smoke	Effect
Tar	Carcinogenesis
Polycyclic aromatic hydrocarbons	
Nitrosamine	
Benzopyrene	
Nicotine	Tumor promotion
Phenol	

- **Epidemiologic studies:** Cigarette smoking is associated with not only lung cancer but also carcinoma of the mouth, pharynx, larynx, esophagus, pancreas, uterine cervix, kidney and urinary bladder.
- **Clinical evidence:** Microscopic changes in the lining epithelium of the respiratory tract in habitual smokers also revealed the carcinogenic effect of smoking. There is a **positive relation between the intensity of exposure to cigarette smoke and the histological changes in the respiratory epithelium**. The sequence of changes is: Basal cell hyperplasia → squamous metaplasia → dysplasia → carcinoma in situ → invasive carcinoma.
- **Experimental work:** Few animals developed bronchioloalveolar carcinomas after exposure to tobacco smoke.

Second hand smoke or environmental tobacco smoke and passive smoking also carry a small risk of lung cancers.

Tobacco smoking in lung cancer: Evidence

1. Statistical
2. Epidemiological
3. Clinical
4. Experimental.

Industrial Hazards

- **Ionizing radiation:** High-dose of ionizing radiation is carcinogenic. Increased incidence of lung cancer was reported among survivors of the Hiroshima and Nagasaki atomic bomb blasts. **Radon** is a radioactive gas and indoor air pollution by radon **is associated with increased lung cancer in miners exposed to high concentrations**.
- **Uranium:** It is **weakly radioactive** and uranium miners (both nonsmokers and smokers) have higher incidence of lung cancer than in the general population.
- **Asbestos:** Exposure **increases the risk** and risk increases when associated with smoking. It develops after a latent period of about 10–30 years.
- **Other carcinogens:** Chloromethyl ethers, chromium, nickel, vinyl chloride, polyaromatic hydrocarbons, cadmium, formaldehyde and dioxin.

Others

- **Atmospheric pollution:** The risk of **lung cancers is higher in urban areas** than rural areas, suggesting role of air pollution. Major air pollutants include polycyclic hydrocarbons from fossil fuels and motor vehicle exhaust especially diesel smoke.
- **Diet:** **Vitamin A deficiency** → leads to squamous metaplasia → increased **susceptibility to cancer**. Folate, carotenoid-rich fruits and vegetables, vitamin E and

beta-carotene are associated with reduced risk of lung cancer.

- c. **Idiopathic pulmonary fibrosis:** It is associated with increased risk of lung cancer.
- d. **Heredity:** Epidemiological studies have shown lung cancers in close relatives. First-degree relatives of patients with lung cancer have a two- to six-fold increase in the risk for lung cancer. Various susceptibility genes associated with lung cancers have been identified.
- e. **Genetic predisposition:** Genetic polymorphisms involving cytochrome P-450 gene CYP1A1 → increased capacity to metabolize procarcinogens present in the cigarette smoke → increased risk of lung cancer.

Clinical subgroups of lung cancer: Lung carcinomas can be divided into two clinical subgroups on the basis of likelihood of metastases and response to therapies.

- **Small cell lung carcinomas/(SCLC) (highly metastatic, high response to chemotherapy).**
- **Non-small cell lung carcinomas/(NSCLC) (less metastatic, less responsive).**

Molecular Genetics

Most lung cancers develop as **multistep** process by accumulation of oncogenic “driver” mutations. This causes neoplastic transformation of pulmonary epithelial cells from a premalignant lesion to frank cancer after a number of years. Exposures to **various carcinogens produce sequential genetic alterations and epigenetic changes** that result in the loss of normal control of cell growth which eventually produce neoplasms.

Squamous Cell Carcinoma

Strongly associated with smoking and genetic aberrations include:

- **Inactivation of tumor suppressor gene:** Chromosome deletions involves tumor suppressor loci and involve 3p, 9p (site of the *CDKN2A* gene), and 17p (site of the *TP53* gene). They occur during early stages in tumor evolution. Loss of retinoblastoma (RB) tumor suppressor is found in 15% of squamous cell carcinomas. The cyclin-dependent kinase inhibitor gene *CDKN2A* is inactivated and its protein product, p16, is lost in 65% of tumors.
- **Activation of oncogenes:** These are found both in squamous cell carcinoma and adenocarcinomas.
 - **Amplification of *FGFR1* and *MET*:** Many squamous cell carcinomas have amplification of *FGFR1*, a gene encoding the fibroblast growth factor receptor tyrosine kinase.

- **Mutations:** e.g. *EGFR* (tyrosine kinase domain of this gene), *KRAS*, *BRAF* and *ERBB2*. *EGFR* mutations are more common in adenocarcinomas in nonsmokers.
- **Translocations:** e.g. *ALK*, *ROS1* and *RET*.

Small Cell Carcinoma

Strongest association with smoking and has many molecular features of squamous cell carcinoma. This includes:

- **Inactivation of tumor suppressor gene:**
 - Loss-of-function mutations involving *TP53* (75–90% of tumors).
 - *RB* (almost 100% of tumors).
 - Chromosome 3p deletions.
- **Activation of oncogenes**
 - **Amplification of genes of the MYC family** occurs in 10–40% of small cell carcinomas but is rare in other types.

Adenocarcinoma

It shows oncogenic gain-of-function mutations involving components of growth factor receptor signaling pathways. All are found in a minority of tumors and include:

Activation of Oncogenes

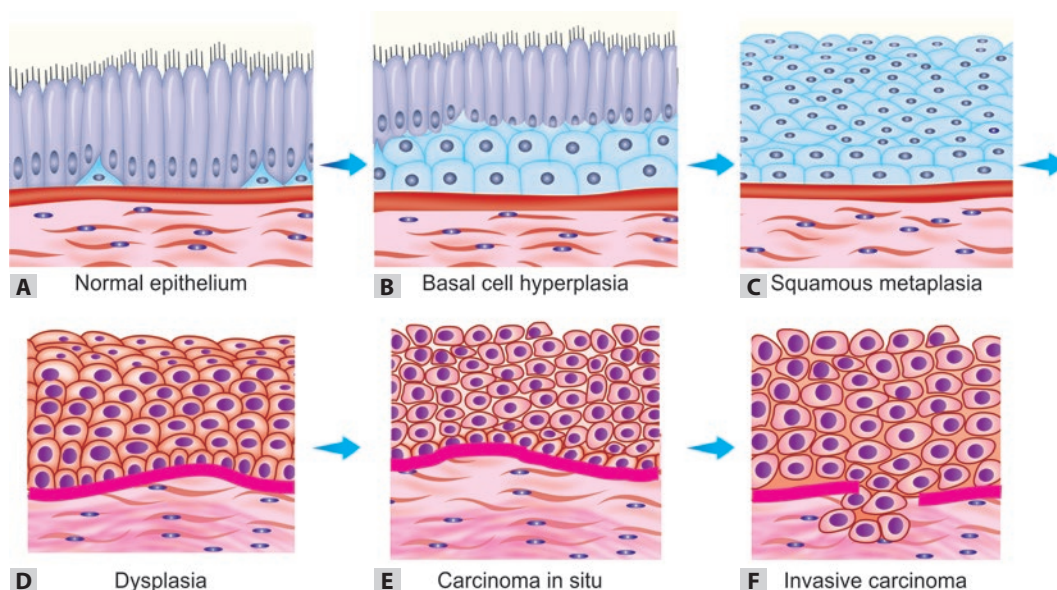
- **Gain-of-function mutations** in multiple genes encoding receptor tyrosine kinases. E.g. *EGFR*, *BRAF*, *ERBB2/HER2*, *ALK*, *ROS*, *MET*, and *RET*, which are also mutated in other types of cancer.
 - **ALK receptor alterations:** Activating rearrangements of the *ALK* gene by fusion with *EML4* gene is observed in 3–7% of adenocarcinoma of lung.
- **Mutations in the *KRAS* gene:** Tumors without tyrosine kinase gene mutations often have mutations in the *KRAS* gene, which is involved in growth factor signaling pathways of receptor tyrosine kinases.

Epigenetics

Precursor epithelial lesions in the lung

1. Squamous dysplasia and carcinoma in situ
2. Atypical adenomatous hyperplasia
3. Adenocarcinoma in situ
4. Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia.

Sequential changes during the pathogenesis of lung cancer are shown in Figure 16.28.



Figs 16.28A to F: Sequential changes during the pathogenesis of lung cancer. (A) Normal epithelium; (B) Basal cell (or reserve cell) hyperplasia; (C) Squamous metaplasia; (D) Dysplasia (characterized by the presence of disordered squamous epithelium, with loss of nuclear polarity, nuclear hyperchromasia, and pleomorphism) may progress through the stages of mild, moderate, and severe dysplasia; (E) Carcinoma-in-situ (CIS); (F) Invasive squamous carcinoma

Classification (Box 16.5)

Q. Write short note on classification of lung cancer.

MORPHOLOGY

Q. Write short note on morphology of lung cancer.

Site (Fig. 16.29)

- **Central (hilar):** Lung cancers arising in and around the hilus of the lung are the most common. About 75% arise from first, second, and third-order bronchi.
- **Peripheral:** Lung cancers may also arise in the periphery of the lung from the alveolar septal cells or terminal bronchioles. They are **usually adenocarcinomas, including adenocarcinoma in situ.**

Centrally (hilar) located lung cancer: Squamous cell carcinoma and small cell carcinoma.

Peripherally located lung cancer: Adenocarcinoma.

Squamous Cell Carcinomas

Preneoplastic Lesions

They **may occur** in the bronchial epithelium and include **squamous metaplasia or dysplasia**, which then transforms to **carcinoma in situ**. They often precede for years and may progress to invasive squamous cell carcinoma.

BOX 16.5: WHO classification of lung tumors

Epithelial tumors

- Squamous cell carcinoma
- Adenocarcinoma
 - Preinvasive lesions: Atypical adenomatous hyperplasia, adenocarcinoma in situ (AIS:non-mucinous/mucinous)
 - Minimally invasive adenocarcinoma (MIA:non-mucinous, mucinous)
 - Invasive: Lepidic, acinar; papillary, micropapillary, solid (according to predominant pattern)
- Neuroendocrine tumors
 - Small-cell carcinoma: Combined small cell carcinoma
 - Large-cell neuroendocrine carcinoma: Combined large-cell neuroendocrine carcinoma
 - Carcinoid tumors: Typical, atypical
- Large-cell carcinoma
- Adenosquamous carcinoma
- Carcinomas with pleomorphic, sarcomatoid, or sarcomatous elements
- Salivary gland type tumors
- Benign tumors: Squamous papilloma, adenoma

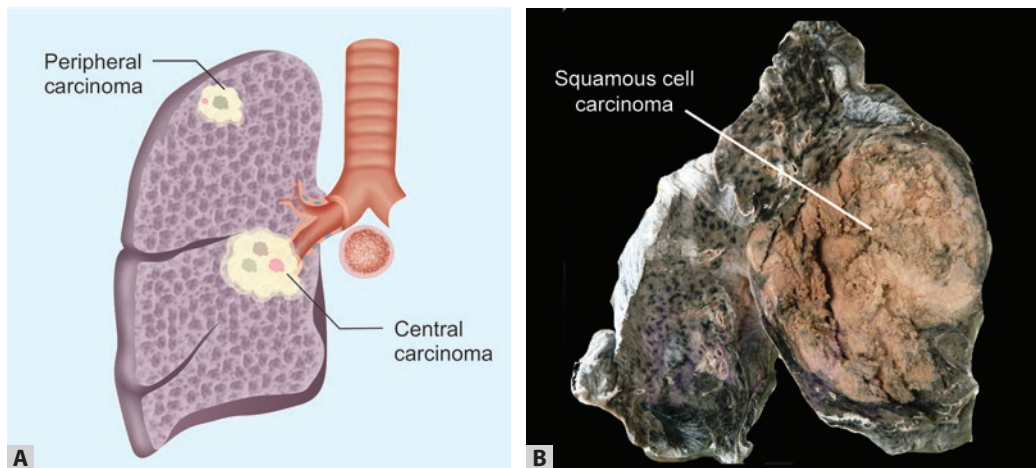
Mesenchymal tumors

- Pulmonary hamartoma
- Chondroma
- PEComatous tumors

Lymphohistiocytic tumors

Tumors of ectopic origin

Metastatic tumors



Figs 16.29A and B: (A) Gross types of carcinoma lung namely, central and peripheral (diagrammatic); (B) Advanced expansile squamous cell carcinoma of lung occupying both central and peripheral region and pushes the surrounding lung substance

Squamous cell carcinoma is **most common in men** and is **most strongly related to cigarette smoking**.

Squamous cell carcinoma of lung:

- Most common lung cancer in smokers
- Most common lung cancer in males.

MORPHOLOGY

Gross (Fig. 16.29)

- **Site and pattern of growth:** Most tumors arise in the **central portion of the lung** from the major bronchi (main to segmental bronchus).
 - **Peribronchial growth:** In which the tumor appears as ulcerated lesions in the bronchi that penetrates the wall of the involved bronchus and infiltrate along the peribronchial into the adjacent carina or mediastinum.
 - **Intraparenchymal growth** (Fig 16.29): In this pattern, the tumor grows like a cauliflower-like intraparenchymal mass which pushes the surrounding lung substance.
 - **Exophytic growth:** It starts as endobronchial tumor projecting into the lumen of the bronchi producing an intraluminal mass. As it enlarges, it obstructs the involved bronchus leading to atelectasis and infection distal to the obstruction.
- **Color and consistency:** Gray-white in color and firm to hard in consistency.
- **Cut section:** It may show focal areas of hemorrhage or necrosis. It may produce red or yellow-white mottling and softening. The central necrotic foci may form cavity.

Grades of squamous cell carcinoma: Well differentiated; Moderately differentiated; Poorly differentiated.

Microscopy (Fig. 16.30)

The characteristic features of squamous cell differentiation are:

- **Keratinization:**

- **Epithelial keratin pearls** or
- **Individual cell keratinization.**

- **Intercellular bridges:** They are seen as slender gaps between adjacent cells.
- **Grades:** On the basis of the amount of keratinization graded as:
 1. **Well differentiated:** These tumors show keratinization in the form of **keratin/squamous pearls**. They appear as **small round nests of brightly eosinophilic aggregates of keratin surrounded by concentric (onion skin) layers of squamous cells.**
 2. **Moderately differentiated:** These tumors show **individual cell keratinization**. This appears as cells with intensely eosinophilic, glassy and dense cytoplasm.
 3. **Poorly differentiated:** These tumors may show only intercellular bridges without any foci of keratinization. **Mitotic activity is higher** in these tumors.

Lung cancer: Tumor cells may be detected in the sputum by exfoliative cytology. It can also be diagnosed by cytological examination of bronchoalveolar lavage fluid, or fine-needle aspiration.

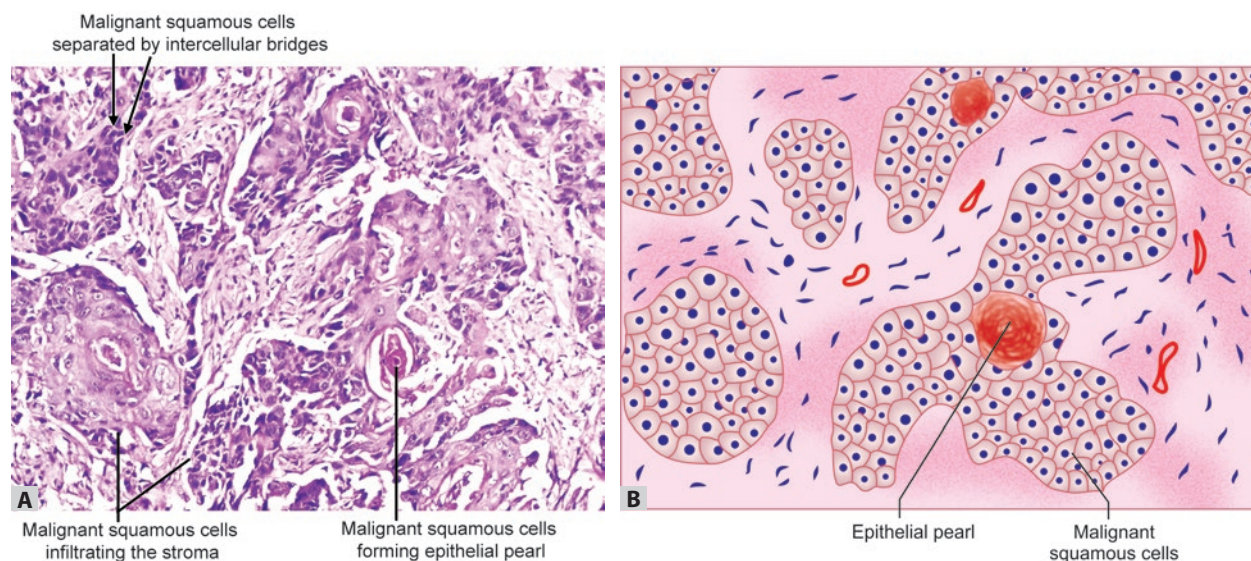
Adenocarcinoma

Adenocarcinoma in situ (AIS)

Adenocarcinoma in situ:

- Tumor 3 cm or less in diameter
- Lepidic growth pattern
- No stromal invasion

Once known as bronchioloalveolar carcinoma (BAC-obsolete) has distinct gross, microscopic, and clinical features. It is a preinvasive form of adenocarcinoma.



Figs 16.30A and B: (A) Photomicrograph; (B) diagrammatic—Squamous cell carcinoma of lung

MORPHOLOGY

Gross

- Occurs in the **terminal bronchioalveolar regions** of the lung.
- Seen at the **peripheral portions of the lung** as a **single nodule** (coin lesion) measuring 3 cm or less.

Microscopy (Fig. 16.31)

- Shows a **pure bronchioalveolar growth pattern** with **no evidence of stromal, vascular, or pleural invasion**.
- Characteristically, they **grow along pre-existing alveolar walls without destruction of alveolar architecture**. This growth pattern is known as **lepidic**, where the neoplastic cells resemble butterflies sitting on a fence.
- **Subtypes**
 - **Nonmucinous:** It consists of columnar, peg-shaped, or cuboidal cells.
 - **Mucinous:** It consists of distinctive, tall, columnar cells with cytoplasmic and intra-alveolar mucin, growing along the alveolar septa.
 - **Mixed:** Mixture of both nonmucinous and mucinous type.

Minimally Invasive Adenocarcinoma

In an adenocarcinoma showing lepidic growth, presence of a minimal invasion does not adversely affect prognosis. This category of adenocarcinoma is termed as minimally invasive adenocarcinoma (MIA, formerly bronchioalveolar carcinoma—obsolete) and has the same favorable prognosis as AIS. MIA is defined as a lepidic- predominant adenocarcinoma tumor as in AIS, but with foci of invasion less than or equal to 5 mm in maximal dimension and without any pleural or lymphovascular invasion and necrosis. At the site of tumor there may be pleural fibrosis and subpleural scars.

These scars represent desmoplastic response to the tumor. These tumors are single and discrete and usually non-mucinous.

Invasive Adenocarcinoma

Adenocarcinoma of lung: Precursor lesion may be atypical adenomatous hyperplasia (AAH).

Adenocarcinoma is an invasive malignant epithelial tumor in which tumor cells show glandular differentiation or mucin production. Most **common type of carcinoma in women and nonsmokers**. They are classified based on the predominant growth pattern namely: lepidic, acinar, papillary, solid and micropapillary.

Adenocarcinoma of lung:

- Overall most common type of cancer
- Most common lung cancer in non-smokers and females

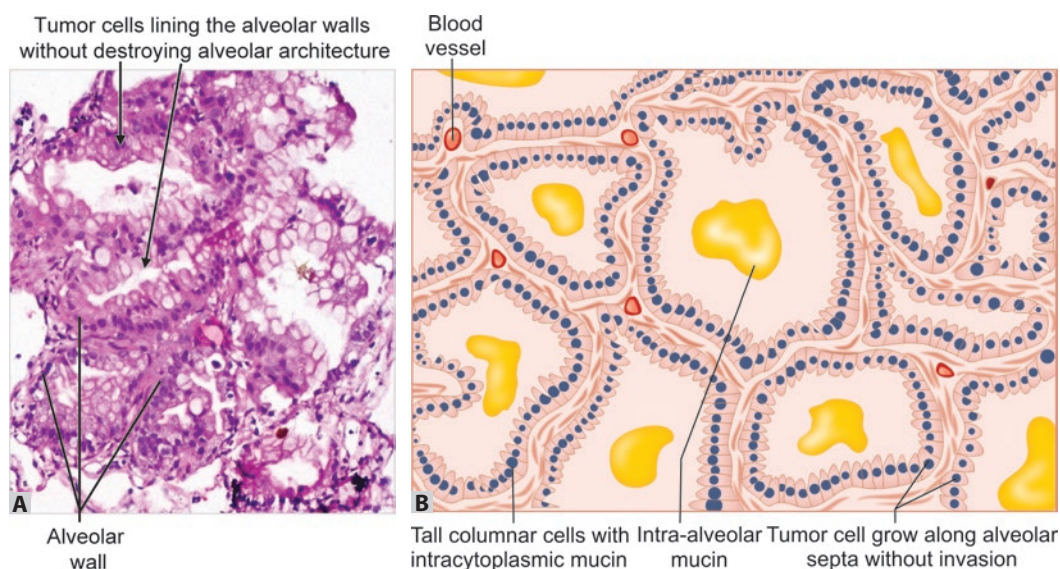
MORPHOLOGY

Gross

- **Site:** Usually more **peripherally located** (in contrast to squamous cell carcinoma), and **tend to be smaller**.
- **Appearance:** They appear as **irregular masses** ranging from 2 to 5 cm in diameter.
- **Cut section:** Nonmucinous tumors appear **grayish white**. Mucinous tumors may appear glistening or gelatinous (depending on the amount of mucus).

Microscopy

Most adenocarcinomas show mixtures of lepidic, acinar, papillary, micropapillary and solid patterns: (i) **lepidic**—predominant tumors show prominent lepidic growth pattern, (ii) **acinar**—predominant



Figs 16.31A and B: (A) Photomicrograph; (B) Diagrammatic. Adenocarcinoma in situ

pattern tumor consists of regular glands lined by cuboidal or columnar cells, (iii) **papillary** adenocarcinomas show a single cell layer covering a fibrovascular connective tissue core, (iv) **micropapillary** carcinomas consist of small papillary tufts of tumor cells without fibrovascular core, (v) **solid** adenocarcinomas with mucus formation are poorly differentiated tumors.

Mucinous adenocarcinomas consist of tall columnar cells with apical cytoplasmic mucin. They usually spread aerogenously and form satellite tumors. Grossly, they may appear as solitary nodule or as multiple nodules, or an entire lobe may be consolidated by tumor (resembles lobar pneumonia).

Majority of tumors are **positive for thyroid transcription factor-1 (TTF-1)**.

Spread: They grow more slowly than squamous cell carcinomas but metastasize widely and earlier.

Neuroendocrine Tumors

Neoplasms of neuroendocrine cells in the lung include (i) low-grade carcinoids and (ii) the highly aggressive small cell carcinoma and large cell neuroendocrine carcinoma of the lung.

Small Cell Carcinoma (SCLC)

Q. Write short note on small cell carcinoma of lung.

- Previously known as oat cell carcinoma.
- **Highly malignant** epithelial tumor with neuroendocrine features and is **strongly associated with cigarette smoking**. The male-to-female ratio is 2:1.

Small cell carcinoma of lung: There is no known preinvasive phase or carcinoma in situ.

Small cell carcinoma:

- Commoner in smokers
- High expression of Bcl-2 gene.

MORPHOLOGY

Gross

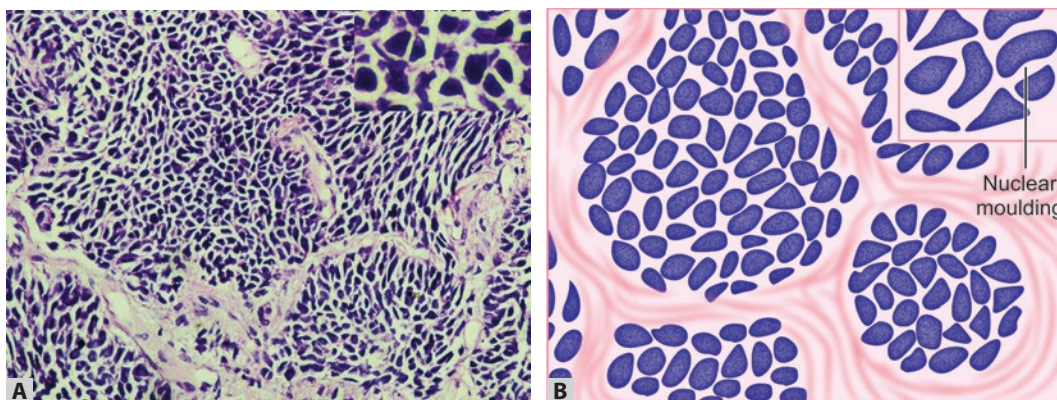
- **Site:** It may arise not only in **major bronchi (central)** but also in the **peripheral portion** of the lung.
- **Cut section:** It is **soft, friable, and white** and shows **extensive hemorrhage and necrosis**.

Small cell carcinoma of lung:

- May be central or peripheral
- Most commonly associated with paraneoplastic syndromes.

Microscopy (Fig. 16.32)

- **Characteristics of tumor cells:**
 - **Size and shape:** **Small round, to fusiform**. The small cell is termed as an oat cell. There is no absolute size for the tumor cells, but a useful rule of thumb is that their diameter/size is less than three times the diameter of a small resting lymphocyte (a size of about 25 microns).
 - **Cytoplasm:** **Scanty**, faintly stained with ill-defined cell borders.
 - **Nucleus:** It is hyperchromatic with **finely granular chromatin (salt and pepper pattern)** and **nuclear molding** (better seen in cytology specimens) is prominent.



Figs 16.32A and B: Microscopic appearance of small cell carcinoma. Inset shows nuclear moulding. (A) Photomicrograph; (B) Diagrammatic

- **Nucleolus:** Absent or **inconspicuous**.
- **Mitotic count:** **High** with an average of 60–70 mitosis per 10 high-power fields.
- **Cells are fragile** and show fragmentation and **crush artifact** in biopsy specimens.
- **Growth pattern:** The tumor cells grow in **sheets or clusters**. The stroma is delicate, vascular, and scant.
- **Necrosis:** **Common and extensive**. Blood vessel wall may stain blue due to deposition of DNA from necrotic tumor cells (**Azzopardi effect**).

Small cell carcinoma: Azzopardi effect—blood vessel stain blue due to DNA deposits from necrotic tumor cells.

Electron Microscopy

The tumor cell shows **membrane-bound neurosecretory granules** similar to the neuroendocrine cells.

- **Origin of tumor:** Small cell carcinoma arises from neuroendocrine progenitor cells present in the lining bronchial epithelium. The evidences for this include (i) presence of neurosecretory granules, (ii) expression of neuroendocrine markers (e.g. chromogranin, synaptophysin, and CD57), and (iii) secretion of hormones (e.g. parathormone-related protein which produces paraneoplastic hypercalcemia) by some tumors. Small cell carcinoma is the one of the most common lung cancer associated with ectopic hormone production and paraneoplastic syndromes. Immunohistochemistry shows high positivity of the antiapoptotic protein BCL2 in 90% of tumors.
- **Behavior:** All small cell carcinomas are **high grade** and are most aggressive lung tumors. They **widely metastasize** and are **incurable by surgery** but are **markedly sensitive to chemotherapy**.
- Combined small cell carcinoma is a variant in which small cell carcinoma is mixed with other nonsmall cell tumors (e.g. large cell neuroendocrine carcinoma).

Small cell carcinoma:

- Small cells
- Salt and pepper chromatin
- Nuclear molding
- High mitotic count
- Extensive necrosis
- Neurosecretory granules.

Large Cell Carcinoma

- Large cell carcinoma is an **undifferentiated malignant epithelial tumor** of lung.
- The tumor cells do not have the characteristic of small-cell, glandular or squamous cell carcinoma.
- They probably represent so undifferentiated squamous cell carcinomas and adenocarcinomas that cannot be identified by light microscopy. Its diagnosis is by exclusion.
- Some large cell carcinomas are called large cell neuroendocrine carcinoma. They grow like carcinoid tumors, i.e. organoid pattern, trabecular growth, peripheral palisading of cells and rosette formation. They also show neuroendocrine differentiation by immunohistochemistry or ultrastructure.

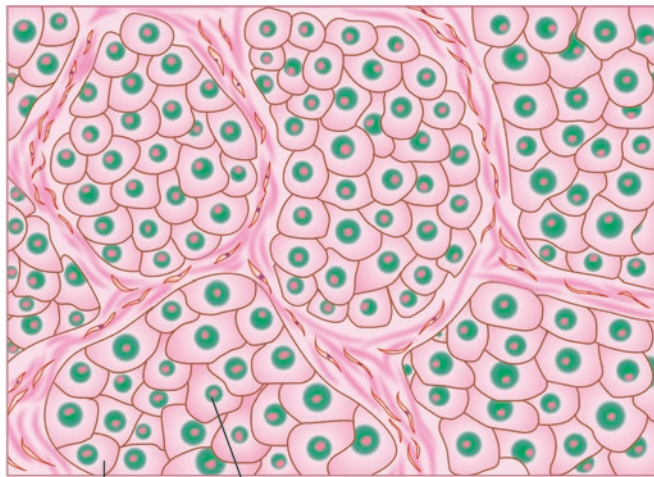
MORPHOLOGY

Gross

- It arises more frequently in the **periphery of the lung**. It forms a spherical tumor with well-defined borders. Cut section shows bulging, fleshy, homogeneous, rather sarcomatous appearance.

Microscopy (Fig. 16.33)

- Tumor cells are large with moderate amount of cytoplasm, large nuclei, prominent nucleoli and vesicular chromatin.



Large cells with abundant cytoplasm
Vesicular nuclei with prominent nucleoli

Fig. 16.33: Large cell carcinoma of lung consisting of large tumor cells with moderate amount of cytoplasm, large nuclei with vesicular chromatin and prominent nucleoli

Combined Carcinoma

It constitute about 10% of all lung carcinomas. Microscopically they have combined histology of two or more of the above histological types. Example: Squamous cell carcinoma and adenocarcinoma or small-cell and squamous cell carcinoma.

Carcinoid Tumors

- Carcinoid tumors are low-grade malignant epithelial neoplasms that arise from the resident neuroendocrine cells normally in the bronchial epithelium.
- Age and gender: Most common below 40 years of age and both males and females are equally affected. There is no relation with cigarette smoking and about 20–40% of patients are nonsmokers.

MORPHOLOGY

Gross

Carcinoids may arise centrally or may be peripheral.

- **Central tumors:** Usually they present as fingerlike or spherical polypoid masses in the mainstem bronchi covered by an intact mucosa. They are small and measure about 3.0 cm diameter. They project into the bronchial lumen as an endobronchial lesion. Some may penetrate the bronchial wall into the peribronchial tissue, producing the so-called collar-button lesion.
- **Peripheral tumors:** are solid and nodular.

Microscopy:

It shows organoid, trabecular, palisading, ribbon, or rosette-like arrangements of cells separated by a delicate fibrovascular stroma. Similar to carcinoids of the gastrointestinal tract, the individual tumor cells are uniform, regular with uniform round nuclei with finely granular chromatin. The cytoplasm is moderate, eosinophilic and finely granular. Depending on the microscopic appearance they are subclassified into:

1. **Typical carcinoids:** These tumors have less than two mitoses per 10 high-power fields and lack necrosis.
2. **Atypical carcinoids:** They differ from typical carcinoids by (i) increased mitoses (two and 10 mitoses per 10 high-power fields) (ii) foci of necrosis, (iii) increased pleomorphism, (iv) increased cellularity, and (v) tendency to grow in a disorganized fashion and invade lymphatics.

Electron microscopy: Tumor cells contain dense-core granules.

Immunohistochemistry: Shows positivity for serotonin, neuron-specific enolase, bombesin, calcitonin, or other peptides.

Clinical Features

Carcinoid tumors grow slowly and about 50% of patients are asymptomatic at presentation. They are often discovered incidentally.

If a patient is symptomatic, symptoms are due to:

- **Intraluminal growth:** Persistent cough, hemoptysis, secondary infections, dyspnea, bronchiectasis, emphysema, and atelectasis.
- **Metastasis and secretion of vasoactive amines:** Majority neither metastasize nor have secretory activity and follow a relatively benign course. In about 10% of cases, they may produce classic carcinoid syndrome, characterized by intermittent attacks of diarrhea, flushing, and cyanosis.

Secondary Changes due to Tumor

They occur in the lung distal to the point of bronchial involvement.

1. **Obstruction of the lumen of a major bronchus.**
 - **Partial obstruction** → may cause **focal emphysema**.
 - **Total obstruction** → may lead to **atelectasis** (collapse of the lung) distal to obstruction.
2. **Suppuration: Due to impaired drainage of the airways.**
 - **Severe suppurative or ulcerative bronchitis or bronchiectasis.**
 - **Pulmonary abscesses.**
3. **Compression or invasion of the superior vena cava:** It produces marked dilation of the veins of the head, neck,

and arms and cyanosis → results in a characteristic clinical complex known as **superior vena cava syndrome**.

SVC syndrome: Most commonly caused by small cell lung cancer.

Spread of Tumor

(Fig. 16.34 and Table 16.6)

Q. Write short note on mode of spread of lung cancer.

Local Spread

- Extension to the **pleural surface**, pleural cavity (**pleuritis**) or into the pericardium (pericarditis) associated with effusions.
- **Tumor at the apex of lung** may invade the **neural structures around the trachea** and the **cervical sympathetic plexus**. They may produce **severe pain in the distribution of the ulnar nerve** and **Horner syndrome** (enophthalmos, ptosis, miosis, and anhidrosis) on the same side as the tumor. **Such tumors** are also called as **Pancoast tumors**.

Horner syndrome: Enophthalmos, lid lag (ptosis), miosis and anhidrosis on the same side of lung cancer.

Horner syndrome: Caused due to compression of sympathetic nerve plexus by apical tumor of the lung called as Pancoast tumors.

Lymphatic Spread

- Tumor may spread into the **tracheal, bronchial, hilar and mediastinal nodes**. It may also spread to lymph nodes in the neck (scalene node) and clavicular region. Involvement of supraclavicular node (**Virchow node**) may call attention to primary.

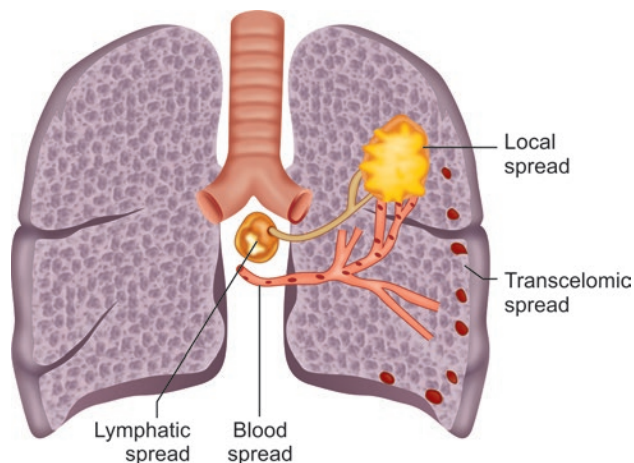


Fig. 16.34: Various routes of spread of lung cancer

Hematogenous Spread

- Common sites of metastases are to **adrenals (in more than 50%)**, **liver**, **brain** and **bone**.

Staging: TNM system is used for staging cancer.

Most common site of metastasis in lung carcinoma: Adrenal.

Clinical Course

- Lung cancer is insidious in onset.

Diagnosis of bronchogenic carcinoma:

- Sputum cytology
- Fiberoptic bronchoscopy
- Bronchial lavage, washings and brushings
- Radiological examination of chest
- FNAC
- Bronchoscopic biopsy.

- Major symptoms include cough, weight loss, chest pain and dyspnea.
- Local effects of lung cancer and their pathologic bases are listed in Table 16.6.
- The tumor may also present with symptoms due to metastasis.

Investigations

- **Radiologic examination** of the chest.
- **Cytologic examination** of sputum, bronchial washings or brushings or fine needle aspiration.

TABLE 16.6: Local effects of lung cancer

Pathological basis	Local effects
• Obstruction of airway by tumor	Pneumonia, abscess, atelectasis, focal emphysema
• Local spread into pleura	Pleuritis and effusion
• Local spread to pericardium	Pericarditis, effusion, tamponade
• Compression of SVC by tumor	Superior vena cava syndrome
Invasion of	
• Recurrent laryngeal nerve	Hoarseness
• Phrenic nerve	Diaphragm paralysis
• Sympathetic ganglia	Horner syndrome
• Esophagus	Dysphagia
• Chest wall	Destruction of rib

Small cell carcinoma of lung:

- Cushing syndrome (due to ACTH)
- Hyponatremia (due to ADH)

Prognosis: Poor for most patients with lung carcinoma. In general, the adenocarcinoma and squamous cell carcinomas remain localized and have slightly better prognosis than undifferentiated carcinomas.

Paraneoplastic Syndromes

Squamous cell carcinoma of lung: Hypercalcemia due to parathormone or parathyroid related peptide.

Lung cancer may occasionally be associated with paraneoplastic syndromes. They are summarized in Table 16.7.

Any histologic types of tumors may produce any one of the hormones. **Usually, ACTH and ADH are produced by small cell carcinomas, whereas hypercalcemia is mostly observed in squamous cell carcinomas.**

Other Systemic Manifestations

- **Lambert–Eaton myasthenic syndrome:** It is characterized by muscle weakness due to auto-antibodies directed against the neuronal calcium channel.
- **Peripheral neuropathy:** It is usually purely sensory.
- **Dermatologic abnormalities:** Acanthosis nigricans.
- **Hematologic abnormalities:** Leukemoid reactions.
- **Hypertrophic pulmonary osteoarthropathy:** It is associated with clubbing of the fingers.

Lambert–Eaton myasthenic syndrome: Muscle weakness due to autoantibodies directed against the neuronal calcium channel.

METASTATIC TUMORS

Q. Write short note on metastatic tumors of lung.

- The lung is the most common site for metastasis.
- Both carcinomas and sarcomas from any site may spread to the lungs via the blood or lymphatics or by direct invasion.

TABLE 16.7: Paraneoplastic syndromes in lung cancer

Elaborated hormones or hormone-like factors	Effects
Antidiuretic hormone (ADH)	Hyponatremia
Adrenocorticotrophic hormone (ACTH)	Cushing syndrome
Parathormone, parathyroid hormone-related peptide	Hypercalcemia
Calcitonin	Hypocalcemia
Gonadotropins	Gynecomastia
Serotonin and bradykinin	Carcinoid syndrome



Fig. 16.35: Lung with multiple secondaries

- Local spread into the lungs occur in carcinoma of esophagus, carcinomas and mediastinal lymphomas.

Metastasis/secondaries: Most common malignant tumor of the lung.

MORPHOLOGY

The pattern of metastatic growth is variable.

- **Multiple nodules** (Fig. 16.35): Usually they appear as multiple **discrete nodules, distributed throughout all lobes**, more being at the periphery. Radiologically, when large nodules are seen in the lungs, they are known as **cannon-ball metastases**.
- **Other patterns:** They may also present as solitary nodule, endobronchial, pleural, pneumonic consolidation, or mixtures of any of these patterns.

Sources of Metastases to Lung

- **Common:** Carcinoma of **gastrointestinal tract, breast, thyroid, kidney, pancreas and liver.**
- **Other tumors:** Osteogenic sarcoma, neuroblastoma, Wilms tumor, melanoma, lymphomas and leukemias.

The microscopic appearance of metastases usually resembles that of the primary tumor.

PLEURAL TUMOR

Malignant Mesothelioma

Malignant mesothelioma is a rare malignant tumor of mesothelial cells.

Sites: Mesotheliomas can arise in the **pleura** (most common), **peritoneum, pericardium**, tunica vaginalis of the testis and genital tract (benign adenomatoid tumor).

Thoracic mesothelioma arises from either the visceral or the parietal pleura.

Etiology

- Exposure to **asbestos**.
- Homozygous **deletion of the tumor suppressor gene *CDKN2A/INK4a*** involving chromosome 9p (in about 80% of mesotheliomas).

MORPHOLOGY

Gross

- Malignant pleural mesothelioma is a **diffuse lesion** that arises either from the visceral or parietal pleura. It usually **spreads widely in the pleural space** and is associated with extensive pleural effusion and direct invasion of thoracic structures.
- It appears as thick layer of soft, gelatinous, grayish-pink tumor tissue that often encase and compress the affected lung. It may also extend into fissures and interlobar septa, a distribution often termed as a “pleural rind”.

Microscopy

Types

1. **Epithelioid type (60%)**: Consists of **cuboidal, columnar, or flattened cells** forming **tubular, glandular or papillary** structures resembling adenocarcinoma. Immunohistochemistry is

essential for differentiating mesothelioma from adenocarcinoma of lung. Most mesotheliomas are strongly positive for keratin proteins, calretinin, Wilms tumor 1 (WT-1), cytokeratin 5/6, and D2-40.

2. **Mesenchymal (sarcomatoid) type (20%)**: Consists of **spindle cells** similar in appearance to a fibrosarcoma. They have lower expression of many of the markers described under epithelioid type mentioned above.
3. **Mixed (biphasic) type (20%)**: Consists of both epithelioid and sarcomatoid patterns.

Clinical Course

- Average age is 60 years.
- Chest pain, dyspnea, recurrent pleural effusions and nonspecific symptoms, such as weightloss and malaise.

Spread

- Direct local invasion of the lung.
- Lymphatic spread to the hilar lymph nodes.
- Hematogenous spread to the liver bones, peritoneum and adrenals.

Prognosis is poor and fifty percent die within 12 months of diagnosis.

Oral Cavity and Salivary Gland Disorders

PRECANCEROUS LESIONS OF ORAL CAVITY

Q. Write short note on premalignant lesions of the oral cavity.

Leukoplakia and Erythroplakia

Q. Write short note on definition and morphology of leukoplakia.

Leukoplakia

Definition: It is defined as a **white patch or plaque, not less than 5 mm in diameter**, that cannot be removed (scraped off) by rubbing and cannot be classified as any other diagnosable disease.

Leukoplakia: White mucosal patch or plaque that can undergo malignant transformation.

If any white patches in the oral cavity can be given a specific diagnosis, it is not a leukoplakia.

- **Sites:** May be seen **anywhere in the oral cavity**. Most common sites are buccal mucosa, floor of the mouth, ventral surface of the tongue, palate, and gingiva.
- **Number:** It may be **single or multiple**.
- **Appearance:** **White patches or plaques** having **sharply defined borders**. Surface may be smooth or wrinkled.

Leukoplakia: Until otherwise proved by microscopic examination, all leukoplakias must be considered precancerous.

Erythroplakia

Erythroplakia: Risk of malignant transformation is more than that of leukoplakia.

- **Less common** and appears as a **red, velvety area within the oral cavity**.
- It remains level with or slightly depressed in relation to the surrounding mucosa.
- **Microscopically**, the **epithelium is atypical** and has an **increased risk of malignant transformation** than leukoplakia.

Intermediate forms, which have the characteristics of both leukoplakia and erythroplakia, are termed **speckled leukoerythroplakia**.

Features of Leukoplakia and Erythroplakia

Q. List the differences between leukoplakia and erythroplakia.

Age and Gender

Both leukoplakia and erythroplakia may be found in adults of any age, but they are usually seen between **40 to 70 years**. Male to female ratio is 2:1.

Etiology

Both have **multifactorial** origin and are associated with use of **tobacco** (cigarettes, pipes, cigars, and chewing tobacco).

MICROSCOPY

Leukoplakia/erythroplakia: Biopsy to rule out dysplasia or carcinoma.

Leukoplakia

- **Surface stratified squamous epithelium** show a spectrum of changes ranging from **hyperkeratosis and acanthosis** to lesions with **variable degree of dysplastic changes** (including carcinoma in situ).
- **Subepithelial region** shows **inflammatory infiltrate of lymphocytes and macrophages**, the intensity of which is proportional to the degree of dysplasia.

Erythroplakia

- **Epithelium** shows **erosions with dysplasia, carcinoma in situ, or frank carcinoma**.
- **Subepithelial region** shows **intense inflammatory reaction and vascular dilatation**, which is **responsible for the reddish clinical appearance**.

Hairy Leukoplakia

- Hairy leukoplakia is a **distinctive oral lesion** and is seen in **immunocompromised patients**.
- About 80% of patients with hairy leukoplakia are infected with the human immunodeficiency virus (HIV) and remaining 20% are seen in patients who are immunocompromised for other reasons (e.g. cancer therapy or transplant immunosuppression).

Etiology: It is due to **Epstein-Barr virus (EBV) infection**.

Site: Located mostly on the **lateral edges/border of the tongue**.

Toluidine blue:

- Basic metachromatic dye with high affinity for acidic components and stains tissues rich in DNA and RNA.
- Stain used to diagnose premalignant lesion (dysplasia) of oral cavity (e.g. lip).
- Detects efficiently and rapidly mitotic figures in paraffin embedded tissues.

MORPHOLOGY

- **Gross:** **White, confluent patches of fluffy ("hairy") thickenings**, which cannot be scraped off.
- **Microscopy:** It shows **hyperparakeratosis and acanthosis** with **"balloon cells"** in the **upper spinous layer** of the epithelium.

SQUAMOUS CELL CARCINOMA

In India, oral cavity cancer is the most common malignant tumor.

Oral cancer: Majority are squamous cell carcinomas.

Etiology

Q. Write short note on etiology of squamous cell carcinoma of oral cavity.

Squamous cell carcinoma (SCC) of oral cavity is **multifactorial disease** usually seen in middle-aged men.

Most common site of cancer of oral cavity:

- Tongue > lip.
- In India: Buccal mucosa > anterior tongue > lower alveolus.

Risk Factors

Most common type of cancer in India: Carcinoma of oral cavity.

1. **Tobacco products and smoking:** Any irritating smoked product increases the risk for tumors of the oral cavity. Nicotine in tobacco and other tobacco leaf components cause cancer. **Carcinogens in tobacco can act as initiators, as well as promoters.** Risk increases with amount and duration of tobacco use. Tobacco may be used either for **smoking** or as **smokeless tobacco**.
 - **Smoking:** It may be in the form of cigarette, beedi, cigar, or pipe smoking, or reverse smoking (smoking a cheroot with the burning end inside the mouth is practiced in certain regions of India). Regular marijuana use has also been associated with oropharyngeal cancer.
 - **Smokeless tobacco:** It is in the form of **betel quid/pan** that contains several ingredients such as areca nut, slaked lime, and tobacco, which are wrapped in a betel leaf. It is commonly used in India and Southeast Asia, and is associated with marked increase in oral cancer. **Betel quid appears to be the major carcinogen.** However, it may also be related to slaked lime and the areca nut. Other methods of tobacco consumption include snuff dipping and tobacco chewing.

Oral cancers are found on the **buccal and gingival** surfaces in the sites **where tobacco products** are held in **contact** with the mucosa for long periods.

2. **Alcohol consumption:** It is another important etiologic factor and act synergistically with tobacco as either a cocarcinogen (increasing the risk) or a promoter (decreasing the lag time). The risk of oral SCC is magnified in individuals who smoke as well as consume alcohol.
3. **Other risk factors:**
 - Radiation exposure and solar actinic radiation (sunlight).
 - Welding, metal refining, diesel exhaust, wood stove, and asbestos exposure.
 - Chronic irritation of the mucosa: It may be due to ill-fitting dentures, jagged teeth, or chronic infections.
 - Vitamin A deficiency and immunosuppression.
 - Poor nutrition.

Role of Oncogenic HPV Virus Infection

High-risk HPV types 16 and 18 and, less commonly low-risk HPV types 6 and 11 have been found in oral carcinomas.

Squamous cell carcinoma of oral cavity: Risk factors include tobacco and alcohol use. It may also be associated with HPV infection.

Inherited Genomic Instability

Family history of head and neck cancer is a risk factor, and is thought to be due to inherited genomic instability.

Patients with head and neck squamous cell carcinoma are at increased risk for second primary malignancy. Typically in the aerodigestive tract (e.g. lung, esophagus).

Risk factors associated with cancer of the head and neck.

- Tobacco products, smoking and areca nut/pan masala
- Alcohol consumption
- Human papillomavirus
- Plummer–Vinson syndrome
- Inherited genomic instability
- Poor nutrition.

Pathogenesis

Field cancerization: Phenomenon of susceptibility of the oral mucosa for multiple primary cancers.

The development of squamous cell carcinoma is a **multi-step process**. It involves sequential activation of oncogenes and inactivation of tumor suppressor genes in a clonal population of cells.

- **Inactivation of the *p16* gene** (about 80% of the cases) → stratified squamous epithelium undergoes hyperplasia/hyperkeratosis.

- **Mutation of the *p53* tumor suppressor gene** is associated with progression of hyperplasia/hyperkeratosis to dysplasia.
- **Amplification and overexpression of the *cyclin D1* gene** constitutively activates cell cycle progression.

MORPHOLOGY

Site

- Anywhere in the oral cavity. Common sites are **lower lip, the ventral surface of the tongue, floor of the mouth**, buccal mucosa, soft palate, and gingiva.

Gross

- **Early stages:** It appears either as raised, firm, pearly plaques or as irregular, roughened, or verrucous areas of mucosal thickening. May be superimposed on a leukoplakia or erythroplakia.
- **Later:** It may appear as **ulcerated and protruding gray white masses with irregular and indurated (rolled) borders**.

Microscopy

- Squamous cell carcinomas (Figs 17.1A and B) range from well-differentiated keratinizing neoplasms to poorly differentiated/anaplastic tumors. However, the histological grading does not correlate with behavior.

Spread

1. **Local:** Tissue involved depends on the primary site.
2. **Lymph node:** The involved site of lymph node depends on the location of the primary tumor. The more anterior the tumor, more is the spread to the cervical nodes. Carcinomas of the base of the tongue and oropharynx metastasize to the deep retropharyngeal lymph nodes.
3. **Blood spread:** It spreads to lungs, liver, and bones.

Verrucous Carcinoma

Verrucous carcinoma (Ackerman tumor):

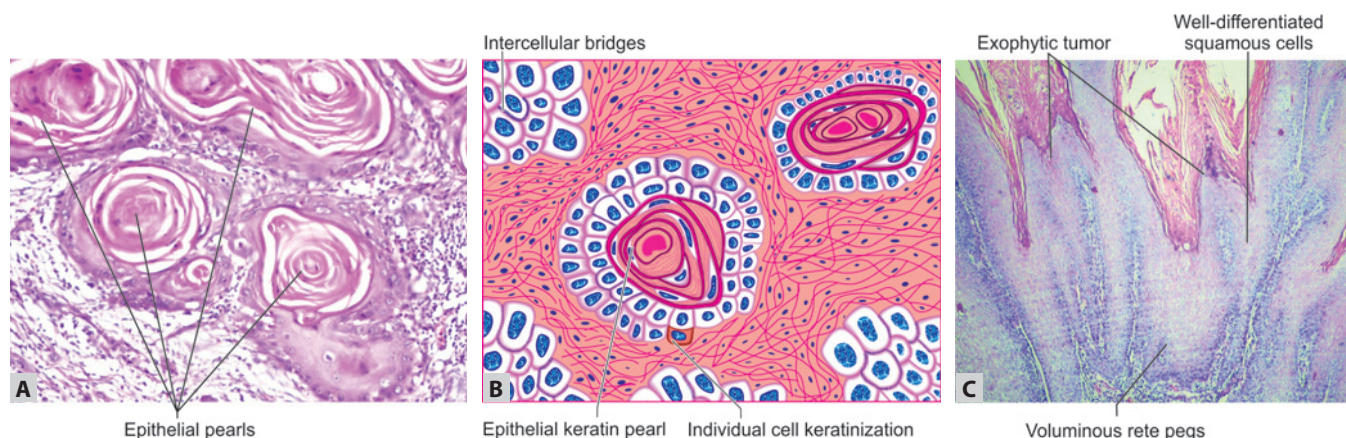
- Distinct variant of well-differentiated squamous cell carcinoma
- Mainly in elderly patients
- Warty appearance
- Pushing infiltrative margins
- Lymphatic spread rare.

Verrucous carcinoma (Ackerman tumor) is a **variant of well-differentiated squamous cell carcinoma**.

Site: Most common in the buccal mucosa and lower gingiva.

Age and gender: Most are elderly males.

Etiology: Tobacco, especially chewing or snuff dipping and HPV infection.



Figs 17.1A to C: Well differentiated squamous cell carcinoma microscopic appearance (A. H and E, B. Diagrammatic); C. Verrucous carcinoma showing exophytic growth, swollen and voluminous rete pegs extending into the deeper tissues

MORPHOLOGY

Gross: Large, fungating, soft papillary growth.

Microscopy (Fig. 17.1C): The surface epithelium shows hyperkeratosis, acanthosis, and benign-appearing papillomatosis. Rete pegs are swollen and voluminous and extend into the deeper tissues.

Spread: May infiltrate the soft tissues of the cheek, mandible or maxilla, and invade perineural spaces. **Regional lymph node metastases are exceedingly rare, and distant metastases have not been reported.**

SALIVARY GLAND NEOPLASMS

Salivary gland neoplasms are relatively uncommon.

Incidence

Parotid gland: Most common site for tumors of salivary gland.

- Parotid gland—65–80% and ~15–30% parotid tumors are malignant
- Submandibular gland—10% and ~40% are malignant.
- Minor salivary glands including the sublingual glands—10%. About 50% of minor salivary gland and 70–90% of sublingual tumors are malignant.
- Malignancy in salivary gland is inversely proportional to the size of the gland.

Classification of Salivary Glands Tumors (Table 17.1)

Q. Classify salivary gland tumors.

- **Age:** Benign tumors occur in the fifth to seventh decades of life. The malignant tumors appear later.

TABLE 17.1: Classification of tumors of the salivary glands

Benign	Malignant
Pleomorphic adenoma (mixed tumor)	Mucoepidermoid carcinoma
Warthin tumor	Adenocarcinoma
Oncocytoma	Acinic cell carcinoma
Basal cell adenoma	Adenoid cystic carcinoma
	Malignant mixed tumor

- **Sex:** Slight female predominance, except Warthin tumor, which occur often in males than in females.

Clinical Presentation

- Parotid gland neoplasms produce swellings in front of and below the ear.
- Both benign and malignant tumors range in size from 4–6 cm in diameter.
- Most tumors are mobile except advanced malignant tumor.

PLEOMORPHIC ADENOMA

Q. Write short note on pleomorphic adenoma.

Pleomorphic adenoma is a **most common benign tumor of the salivary glands**, characterized by an **admixture of epithelial and stromal elements**, and is also called **mixed tumors**. In pathology, the term "pleomorphic" is used to indicate nuclear variation (size and shape of nuclei) in neoplasm. However, the term pleomorphic adenoma is used because of variable cell type seen in this lesion.

Age: It occurs usually during **third to fifth decade** of life (middle-age).

Sex: It is most frequent in **females**.

Pleomorphic adenoma: Most common benign tumor of the salivary glands.

Pleomorphic adenoma:

- Most common tumor of major salivary gland mainly parotid
- Less common in submandibular and sublingual gland
- Rare in minor glands.

Site

- **Major salivary gland:** Common site and constitute about 60% of tumors in the parotid. Usually arise in the **superficial lobe of the parotid**. Less common in the submandibular glands and very rare in the sublingual gland.
- **Minor salivary gland:** Its involvement is relatively rare.

Pleomorphic adenoma: Benign salivary gland tumor composed of mixture of:

1. **Epithelial components**
2. **Mesenchymal-like components.**

Etiology

Not known. Exposure to radiation increases the risk.

- **Nature:** It is a benign tumor.
- **Cell of origin:** Histogenesis uncertain. **It was called as mixed tumor**, because of the mixture of epithelial and mesenchymal components. However, it is now considered that the tumor neoplastic cells (epithelial and those which appear mesenchymal), are of either **myoepithelial or ductal reserve cell origin**.

In significant number of these tumors there is chromosomal rearrangements involving

- PLAG1 (gene encoding a transcription factor). This upregulates the expression of a many genes which increase cell growth (e.g. growth factor receptor signaling pathways).
- Pleomorphic adenoma: Most common site is parotid tail (superficial lobe).

MORPHOLOGY

Q. Write short note on gross and microscopy of pleomorphic adenoma.

Gross

- **Shape:** Round.
- **Size:** Ranges from 2.5 to 6 cm in diameter.
- **Consistency:** It depends on the relative amount of epithelial cells and stroma. **Usually rubbery**, resilient mass with a **bosselated surface**.

- **Well-circumscribed/capsulated:** In some, the capsule is not fully developed, and small extensions can be seen protruding into the surrounding salivary gland. This makes enucleation of the tumor difficult.

Pleomorphic adenoma: Encapsulated but pseudopodia (finger-like projections) into the surrounding gland.

- **Cut surface:** It shows **gray-white** with **myxoid and blue glistening, translucent chondroid** (cartilage-like).

Microscopy (Figs 17.2 and 17.3)

Characteristic feature is the **pleomorphic appearance**. Neoplastic cells show varying **mixture of epithelial tissue** component intermingled with **cells showing mesenchymal differentiation**.

- **Epithelial element:** This component consists of **ductal cells and myoepithelial cells**.
 - **Epithelial elements** are arranged in the form of **ducts, acini and irregular tubules**. The ducts are lined by both epithelial (cuboidal to columnar) cells and **surrounded by myoepithelial components** (a layer of deeply chromatic, small myoepithelial cells).
 - May show **strands or sheets** of plasmacytoid or spindled **myoepithelial cells**.
 - Islands of well-differentiated squamous epithelium may also be seen.
- **Mesenchymal-like elements:** The epithelial elements are dispersed within a varying amount of mesenchyme-like background of loose **myxoid tissue**, **islands of hyaline, chondroid (cartilaginous)**, and **mucoid matrix**.

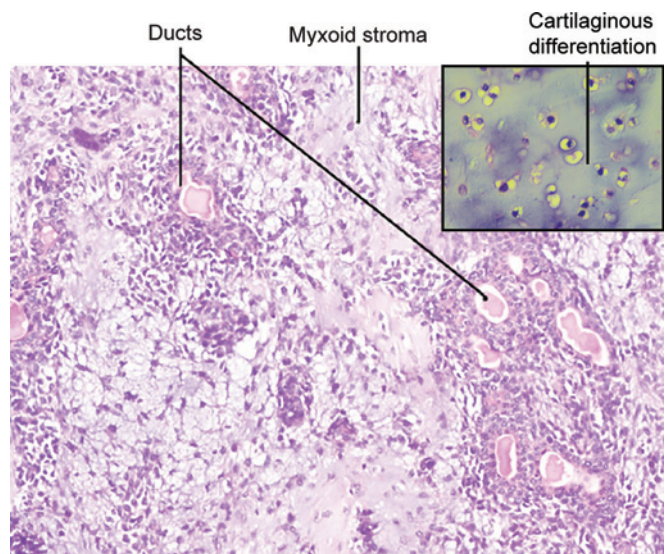


Fig. 17.2: Mixed tumor (pleomorphic adenoma) of the parotid gland shows epithelial cells forming ducts surrounded by myxoid stroma and cartilaginous differentiation inset

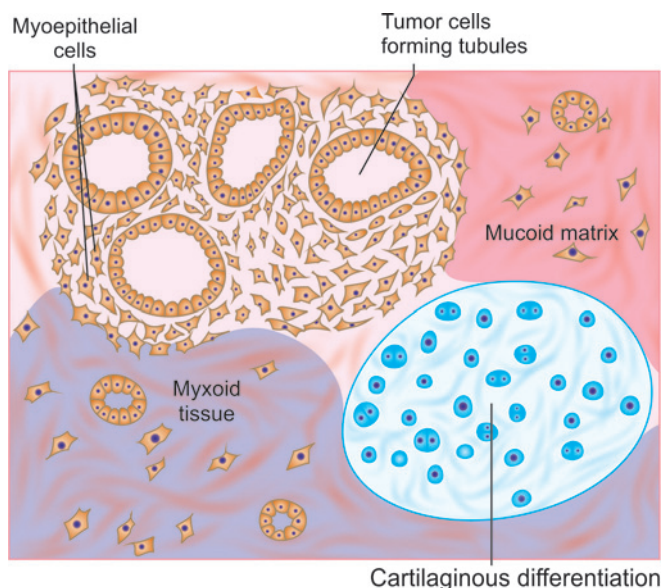


Fig. 17.3: Microscopic features of pleomorphic adenoma of salivary gland (diagrammatic)

Clinical Features

Pleomorphic adenoma: Slow growing tumor.

- Pleomorphic adenomas present as **painless, slow-growing, mobile, discrete** tumors in the **parotid or submandibular** areas or in the buccal cavity.
- The tumors tend to protrude focally from the main tumor into adjacent tissues.

Pleomorphic adenoma: Unicentric but recurrences are multicentric.

- Failure to recognize these minute protrusions at surgery is responsible for recurrence of these tumors.

Carcinoma Ex Pleomorphic Adenoma

- Rarely, a carcinoma may arise in pleomorphic adenomas—referred to as a **carcinoma ex pleomorphic adenoma** or a **malignant mixed tumor**.
- Incidence of carcinoma increases with the duration of the tumor.
- **Microscopy:** These tumors show poorly differentiated adenocarcinoma or undifferentiated carcinoma in an otherwise benign pleomorphic adenoma.

WARTHIN TUMOR

Q. Write short note on Warthin tumor.

Warthin tumor: Exclusively arises in the parotid gland.

Warthin tumor (**papillary cystadenoma lymphomatosum, adenolymphoma**) is a **benign** and the **second most common** salivary gland neoplasm.

- **Site:** It almost **exclusively** arises in the **parotid gland**.
- **Sex:** It is the only salivary glands tumor that is more **common in males** than in females.
- **Age:** It usually occur between **fifth to seventh** decades of life.
- **Predisposing factor:** **Smokers** have eight times the risk of nonsmokers.

Warthin tumor:

- Most common in males
- Fifth to seventh decade
- Associated with smoking.

MORPHOLOGY

Warthin tumor: Second most common benign tumor of the parotid gland.

Gross

- **Shape:** It is round to oval, **encapsulated masses**.
- **Size:** It ranges from **2 to 5 cm** in diameter.
- Usually arises in the **superficial parotid gland** and are readily palpable.
- About 10% are multifocal and 10% bilateral.
- **Cut section:** It is **pale gray** tumor punctuated by narrow cystic or cleft-like spaces filled with a mucinous or serous secretion or even resemble used (dark) motor oil.

Warthin tumor:

- 10% multifocal
- 10% bilateral
- Never involves facial nerve.

Microscopy (Figs 17.4 and 17.5)

Warthin tumor: Consists of

1. Epithelial and
2. Lymphoid elements.

Tumors consist of cystic glandular spaces embedded in dense lymphoid stromal tissue.

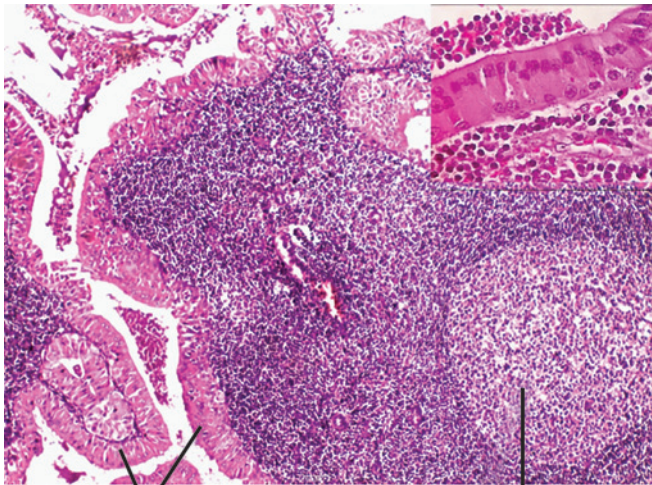
1. Cystic spaces:

- Cystic glandular spaces show **papillary or polypoid projections**.
- Cystic spaces are lined by a distinctive **double layer of neoplastic epithelial cells** consisting of
 - ♦ **Surface superficial layer of columnar cells** with **abundant, finely granular, eosinophilic cytoplasm** (oncocytes). Oncocytes have **abundant mitochondria** → responsible for the **granular appearance** to the cytoplasm.
 - ♦ **Second layer** below the superficial layer consisting of **cuboidal to polygonal cells**.

2. **Lymphoid stromal tissue:** Cystic spaces are embedded in a dense lymphoid stroma which **closely resembles a normal lymph node**. The lymphoid tissue is prominent with germinal centers and is composed of B and few T cells.

Warthin tumor:

1. Cystic spaces are lined by a distinctive double layer of neoplastic epithelial cells
2. Lymphoid stroma.



Cystic spaces lined by double layer of neoplastic cells

Lymphoid stroma with prominent germinal center

Fig. 17.4: Warthin tumor showing epithelial and lymphoid elements. Note the follicular germinal center in the center. The cystic spaces are lined by double layer of eosinophilic epithelial cells (inset) separated by reactive lymphoid stroma

Histogenesis: Warthin tumor may represent an aberrant incorporation of lymphoid tissue in the parotids.

Behavior: It is **benign**, and about 2% may recur.

MUCOEPIDERMOID CARCINOMA

Mucoepidermoid carcinoma is the most common malignant tumor:

- Primary malignant tumor of salivary gland.
- Tumor of salivary gland in children.

Most common primary malignant tumor of the salivary glands.

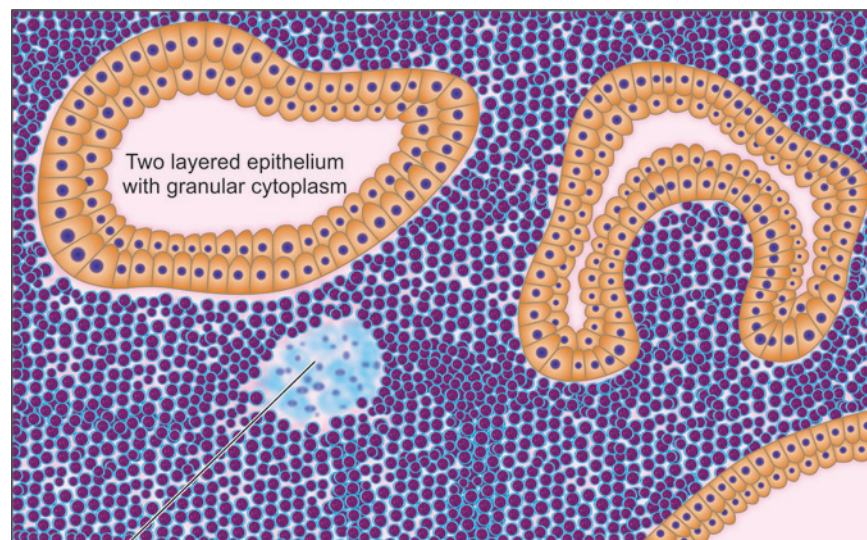
Incidence

- Constitute about 15% of all salivary gland tumors.
- Occur mainly (60–70%) in the parotids.
- Account for a major fraction of the minor salivary gland tumors.

Etiology

In about one-third of cases, it is associated with a distinctive balanced (11;19) (q21;p13) chromosomal translocation. This creates a fusion gene composed of portions of the *CRTC1* (formerly known as *MECT1*) and *MAML2* genes.

The translocation results in activation of the Notch pathway.



Lymphoid follicle with germinal center

Fig. 17.5: Warthin tumor showing epithelial and lymphoid elements (diagrammatic)

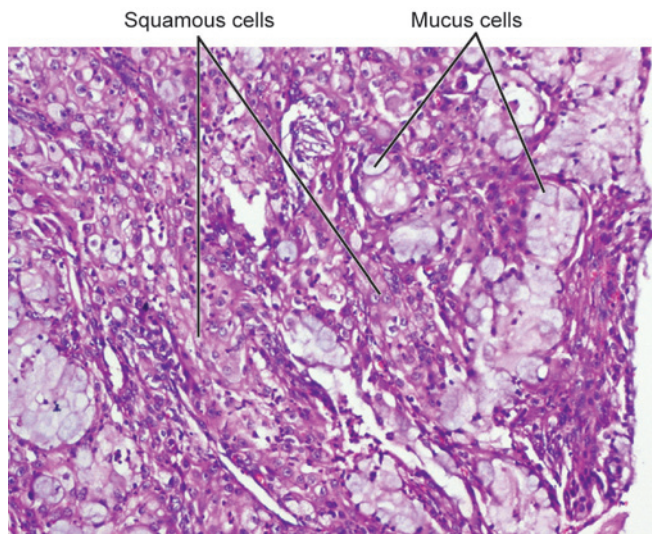


Fig. 17.6: Mucoepidermoid carcinoma composed of squamous cells as well as clear vacuolated cells containing mucin

Mucoepidermoid carcinoma: Malignant neoplasm composed of mixture of:

1. Squamous cells
2. Mucous cells
3. Intermediate cells.

Adenoid cystic carcinoma: Most common malignant tumor of minor salivary glands.

Adenoid cystic carcinoma:

- Cribriform pattern
- Swiss-cheese appearance.

MORPHOLOGY

Gross

- **Size:** It can grow as large as 8 cm in diameter.
- **Appear circumscribed,** but do not show well-defined capsules and are often infiltrative at the margins.
- **Cut section:** It is **pale and gray-white** and frequently contain small, mucin-containing **cysts**.

Tumors that spread perineurally:

1. Adenoid cystic carcinoma
2. Adenocarcinoma of prostate
3. Cholangiocarcinoma
4. Carcinoma of gallbladder
5. Carcinoma of pancreas.

Microscopy (Fig. 17.6)

Composed of variable mixtures of

1. Squamous cells
2. Mucus-secreting cells
3. Intermediate cells.

These tumor cells are arranged in cords, sheets, or cystic structures. The intermediate cells have squamous features, with small to large mucus-filled vacuoles. The mucus stains positive with mucin stains. The tumor cells may appear regular and benign or highly anaplastic and malignant.

Grading: These tumors are graded as low, intermediate or high grade. Clinical features and prognosis depends on the grade of the neoplasm.

Gastrointestinal Tract Disorders

ESOPHAGUS

Esophagus:
Normal length of esophagus: 25–30 cm
Only Auerbach plexus and no Meissner's plexus
Lacks a serosal layer.

Q. Write short note on Mallory-Weiss syndrome.

Lacerations: Mallory-Weiss Tears

Mallory-Weiss tears are nontransmural longitudinal tear in the esophagus at the gastroesophageal region.

Etiology: Associated with **severe/vigorous retching** or **vomiting** secondary to acute alcohol intoxication or vigorous coughing.

MORPHOLOGY

- Site of tears: Usually at the **gastroesophageal junction** but may also be located in the proximal gastric mucosa.
- Appearance of tear: Superficial, **longitudinal oriented lacerations** of **variable** length.

Clinical features: Hematemesis. Usually, do not require surgical intervention, and healing is rapid and complete.

Causes of hematemesis:

1. Mallory-Weiss syndrome
2. Esophageal varices
3. Reflux esophagitis
4. Esophageal ulcers.

Mallory-Weiss syndrome: Longitudinal mucosal tear of distal esophagus.

Barrett Esophagus

Q. Write short note on Barrett esophagus.

Barrett esophagus is characterized by **intestinal metaplasia** within the squamous mucosa of esophagus occurring as a **complication to chronic gastroesophageal reflux (GERD)**.

Incidence: Occur in ~10% of patients with symptomatic GERD.

Gender and age: Males, usually between **40 and 60 years of age**.

Barrett esophagus: Develops in ~10% of patients with symptomatic GERD.

MORPHOLOGY

Gross

Appears as **one or several tongues or patches of red, velvety mucosa** extending from **gastroesophageal junction upwards into esophagus**.

Microscopy (Fig. 18.1)

- **Intestinal metaplasia:** Normal **squamous lining** of the lower esophagus is replaced by **columnar mucosa** (columnar lined esophagus; CLO) containing **areas of intestinal metaplasia**. **Goblet cells** with distinct mucous vacuoles are seen in the region of **intestinal metaplasia**, are **necessary for diagnosis**. **Intestinal metaplasia** is an important **risk factor** for the development of **adenocarcinoma**.
- **Dysplasia** (low grade or high grade): It may be seen in the metaplastic epithelium.

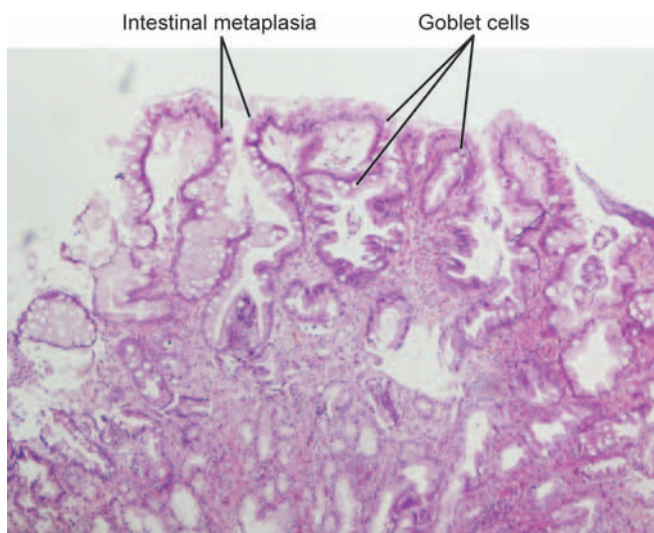


Fig. 18.1: Barrett esophagus with intestinal metaplasia containing goblet cells

Barrett esophagus: Replacement of squamous epithelium by intestinal type of **epithelium with goblet cells** (columnar metaplasia).

Barrett esophagus:

1. Pre-malignant condition
2. Increased risk of esophageal adenocarcinoma.

Complications of Barrett esophagus:

1. High (midesophageal) and long strictures
2. Chronic peptic ulceration (Barrett ulcer)
3. Risk of esophageal adenocarcinoma.

Goblet cells: Characterized by the presence of distinct mucous vacuoles.

ESOPHAGEAL CANCER

Most common site of esophageal cancer: Middle third of esophagus.

Morphologic types: Squamous cell carcinoma and adenocarcinoma.

Squamous Cell Carcinoma

Most common type of esophageal cancer in India is squamous cell carcinoma.

Malignant neoplasm of the esophagus showing squamous differentiation.

Age and gender: Adults over age 45, M:F = 4:1.

Etiology

Q. Write short note on etiology of esophageal cancer.

Risk Factors

- High alcohol intake.
- Tobacco smoking.
- **Nutrition:** Poverty, diet deficient in vitamins and certain trace elements, polycyclic hydrocarbons, nitrosamines, fungal toxins in pickled vegetables foods, etc.
- **Hot beverages:** Frequent consumption of burning-hot beverages, which causes thermal injury.
- **Others:** Caustic esophageal injury, **Plummer-Vinson syndrome**, **achalasia cardia**, celiac disease and **previous radiotherapy** to the mediastinum. **Human papillomavirus (HPV)** infection in high-risk areas but not in low-risk regions.

Squamous cell carcinoma of esophagus: Most common risk factors—

1. Alcohol use
2. Tobacco use.

Plummer-Vinson syndrome:

- Iron deficiency anemia
- Esophageal webs
- Glossitis.

MORPHOLOGY

Gross (Fig. 18.2)

- **Location:** Most common in the **middle third of esophagus**.
- **Patterns of growth:**
 1. **Fungating or exophytic:** Tumor protrude into and obstruct the lumen.
 2. **Ulcerative:** Growth with elevated ulcer edges.
 3. **Infiltrating, or stenotic:** Least common, predominantly intramural.

Mixed gross growth patterns also occur.

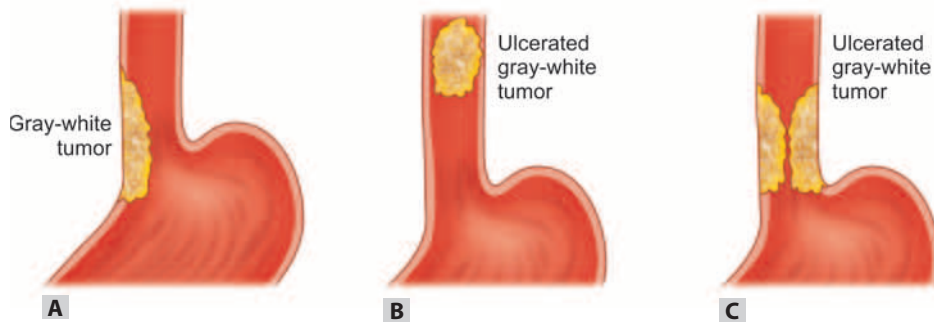
Microscopy (Fig. 18.3)

- Malignant squamous cell with varying degree of differentiation (well to poor differentiated type).

Squamous cell carcinoma of esophagus: Most common site is middle third.

Spread

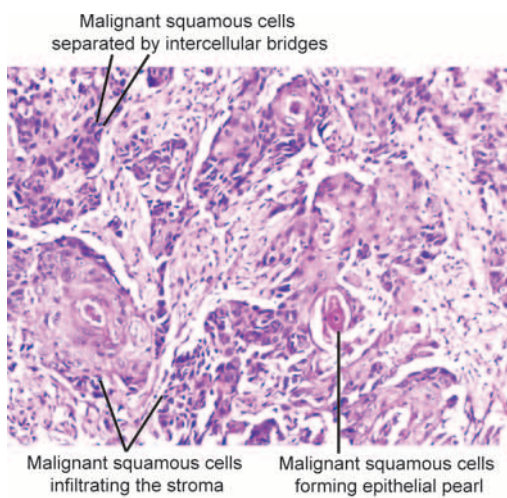
- **Local spread:** Into respiratory tree, aorta, mediastinum and pericardium.
- **Lymphatic spread:** Into regional lymph nodes.
 - Cancers in the upper third to → cervical lymph nodes
 - Middle third to → mediastinal, paratracheal and tracheobronchial nodes
 - Lower third to → gastric and celiac nodes.



Gross types of esophageal cancer: 3 patterns—

1. Fungating
2. Ulcerative
3. Infiltrating.

Figs 18.2A to C: Gross types of carcinoma esophagus: (A) Polypoid type; (B) Ulcerative type; (C) Infiltrative/stenotic type



Squamous cell carcinoma:
Well-differentiated
Moderately differentiated
Poorly differentiated.

Esophagus:

Lymphatic channels in the lamina propria are unique to the esophagus. Dense submucosal lymphatic plexus facilitates early dissemination of esophageal malignancy.

Most common benign tumor of esophagus: Leiomyoma.

H. pylori: CagA positive strain

Protective for adenocarcinoma of esophagus

Can lead to squamous cell carcinoma.

Fig. 18.3: Microscopic appearance of well-differentiated squamous cell carcinoma

- **Hematogenous spread:** Occurs late; to liver, lungs, adrenals, kidneys and bones.

Clinical features: Insidious in onset. Dysphagia, odynophagia (pain on swallowing) and obstruction.

Carcinoma esophagus: Most common symptom—dysphagia > weight loss.

Investigation of choice in esophageal cancer: Endoscopy and biopsy.

Adenocarcinoma

Adenocarcinoma of esophagus: Usually arises in a background of—

1. Barrett esophagus
2. Long-standing GERD.

Carcinoma displaying glandular differentiation and usually arises in a background of Barrett esophagus and long-standing gastroesophageal reflux diseases (GERD).

Etiology

Risk factors include dysplasia, tobacco use, obesity and prior radiation therapy. Risk is reduced by diets rich in fresh fruits and vegetables.

Adenocarcinoma of esophagus: Risk factors—

1. GERD leading to Barrett esophagus
2. Obesity
3. Scleroderma
4. Diet deficient in fruits and vegetables
5. Diet rich in animal fat and cholesterol.

Pathogenesis

Progression of Barrett esophagus to adenocarcinoma develops in a stepwise pattern with genetic and epigenetic changes.

- **Genetic changes** include chromosomal abnormalities and mutation or overexpression of *p53*, amplification of *ERBB-2*, cyclin D1, cyclin E genes, etc.
- **Epigenetic changes** include silencing by hypermethylation of p16/INK4a.

MORPHOLOGY**Gross**

Adenocarcinoma of esophagus: Most common site is lower/distal third.

- **Site:** Usually in the distal third of the esophagus.
- **Appearance:** Early lesions—flat or raised patches → later infiltrate diffusely or ulcerate.

Microscopy

- It consists of malignant tumor with **intestinal-type morphology of cells forming glands**.
- Tumors most commonly produce mucin. Barrett esophagus may be present adjacent to the tumor.

Clinical features: Pain or difficulty in swallowing, chest pain, progressive weight loss, hematemesis, or vomiting.

Barium swallow in esophageal cancer: Rat tail appearance.

STOMACH

Various cells in the mucosa of the stomach and their secretions are presented in Table 18.1.

Definitions

- **Erosion:** Loss of the superficial epithelium which produces a small defect in the mucosa that is limited to the lamina propria (do not penetrate the muscularis mucosae).
- **Ulcer:** Break in the mucosal surface more than 5 mm in size, with depth to the submucosa (i.e. they penetrate the muscularis mucosae). It leads to a local defect or excavation due to active inflammation. **Gastric ulcers (GUs) and duodenal ulcers (DUs)** may be acute or chronic.

TABLE 18.1: Various types of cells in stomach and their secretions

Type of cell	Secretions
Parietal (oxyntic) cells	<ul style="list-style-type: none"> • Acid from the proton pump, H^+-K^+ ATPase • Intrinsic factor required for absorption of vitamin B_{12}
Chief (zymogenic) cells	Proenzymes pepsinogen I and II (activated to pepsin)
Foveolar cells	Mucin layer over the mucosal cells
Endocrine cells	Gastrin in antrum (G cells), histamine in the body

Ulcer: Break in the mucosal surface more than 5 mm in size, with depth to the submucosa.

- **Gastritis: Inflammation of the gastric mucosa** and is usually a histological diagnosis. The term gastropathy is used when inflammatory cells are rare or absent (hypertrophic gastropathy).

Classification of Gastritis (Box 18.1)

BOX 18.1: Various ways of classifying gastritis

A. Depending on the inflammatory cells and duration

- Acute gastritis shows predominately acute inflammatory cells
- Chronic gastritis shows mononuclear cell (lymphocytes, plasma cells) infiltration
 - *Helicobacter pylori* gastritis
 - Autoimmune gastritis
 - Others

B. Depending on the segment of involved stomach

- Antral-predominant gastritis
- Corpus-predominant gastritis
- Pangastritis

C. Depending on the absence or presence of premalignant stages

- Nonatrophic
- Atrophic gastritis → may progress to carcinoma

ACUTE GASTRITIS

Q. Discuss the etiology, pathogenesis and complications of acute gastritis.

Acute gastritis is a **transient inflammation of gastric mucosa**.

Etiology

Acute gastritis: Transient inflammation of gastric mucosa.

- Drugs: Aspirin, nonsteroidal anti-inflammatory drugs (NSAIDs) and other drugs (e.g. iron preparations)
- *H. pylori*
- Alcohol
- Chemicals
- Severe physiological stress (e.g. burns)
- Bile reflux (e.g. following gastric surgery)
- Viral infections (e.g. cytomegalovirus—CMV).

Acute hemorrhagic gastritis: NSAIDs and alcohol

Pathogenesis

Acute (also chronic gastritis) can **develop following disruption of any of the protective mechanisms** (refer page 482-483).

- **Reduced synthesis of mucin and bicarbonate secretion** (e.g. during old age)
- NSAIDs → interfere with protection provided by prostaglandins or reduce bicarbonate secretion.
- **Gastric injury:** E.g. in uremia and infection with urease-secreting *H. pylori* may be due to **inhibition of gastric bicarbonate transporters**.
- **Ingestion of chemicals** (e.g. acids or bases), either accidentally or as a suicide attempt → **direct injury to mucosal epithelium**. Direct cellular injury may also be caused by excessive alcohol consumption, NSAIDs, radiation therapy and chemotherapy.
- **Insufficient epithelial regeneration** following cancer chemotherapy.
- **Decreased oxygen supply** at high altitudes.

MORPHOLOGY

- **Mild acute gastritis:** No significant changes
- **Severe gastritis:** Severe mucosal damage, erosions and hemorrhage → termed **acute erosive hemorrhagic gastritis**.
- **Microscopy:** It shows, dense infiltration by neutrophils within the mucosa and purulent exudate in the lumen.

Gastritis: Presence of neutrophils above the basement membrane in direct contact with the epithelial cells indicates active inflammation.

Clinical Features

May be asymptomatic or cause variable degrees of epigastric pain, nausea and vomiting. When severe, it causes mucosal erosion, ulceration, hemorrhage, hematemesis and melena.

Mucosal Disease Related To Stress

Mucosal disease related to stress develops in association with severe stress. These include severe trauma, extensive burns, intracranial disease, major surgery and any severe physiologic stress.

Types

- **Stress ulcers:** They occur with **shock, sepsis or severe trauma**.

- **Curling ulcers:** They develop in the proximal duodenum with **severe burns or trauma**.
- **Cushing ulcers:** They develop in the stomach, duodenum, and esophagus in patients with **intracranial disease**. Highly prone for perforation.

Curling ulcer: Associated with severe burns.

Cushing ulcer: Associated with head injury/intracranial disease.

Pathogenesis

Mostly due to local ischemia.

- Ischemia due to systemic hypotension or reduced blood flow.
- **Ulcer associated with intracranial injury:** They are probably due to the direct stimulation of vagal nuclei → causes increased secretion of gastric acid.
- **Stress-induced splanchnic vasoconstriction** → hypoxia and reduced blood flow.
- Upregulation of inducible NO synthase and increased vasoconstrictor endothelin-1.

MORPHOLOGY

Gross

- **Size and shape:** It ranges from **shallow erosion** to deep ulcers up to the mucosa; measure less than 1 cm in diameter and round in shape.
- **Site:** **Anywhere** in the stomach.
- **Number:** Single or multiple (more common).
- **Characteristics of ulcer:** Margins and base are not indurated. Floor appears brown to black due to acid digestion of extravasated blood.

Microscopy

- Sharply demarcated, surrounded by normal mucosa
- **Acute inflammatory reaction**
- Complete healing occurs after the injurious agent/factors are removed.

Clinical Features

Most critically ill patients in intensive care units show microscopic evidence of gastric mucosal damage.

CHRONIC GASTRITIS

Chronic gastritis is **chronic** inflammation of stomach associated with mucosal injury. Microscopically, there is an increase in inflammatory cells in the lamina propria.

Causes: Common cause of chronic gastritis is due to infection by *Helicobacter pylori*. Other causes include autoimmune gastritis and less common causes such as radiation injury, chronic bile reflux and mechanical injury (e.g. an indwelling nasogastric tube).

H. pylori Gastritis

Q. Write short essay/note on *H. pylori* gastritis.

- *H. pylori* are short, spiral-shaped or curved (Fig. 18.4) gram-negative bacilli with multiple flagella at one end. They reside on the mucin of the surface and neck regions of foveolar cells. In gastric biopsies examined, they are found in all patients with duodenal ulcers and in majority with gastric ulcers or chronic gastritis.
- Acute *H. pylori* infection usually does not produce noticeable symptoms and produces symptoms when chronic gastritis develops. *H. pylori* can be demonstrated in about 90% of patients with chronic gastritis of the pyloric antrum.
- **Predisposing factors** for *H. pylori* infection includes poverty, overcrowding and limited education. Humans are the primary carriers of infection and transmission is mainly through the fecal-oral route.
- **Mode of spread:** Oro-oral or feco-oral route by either kissing or ingestion of contaminated vomitus.

Pathogenesis

- **Antral gastritis:** *Helicobacter* gastritis is predominantly localized and mostly affects the antrum causing antral gastritis. There may be normal or increased acid

production. If inflammation is limited to the antrum, increased production of acid is likely to cause duodenal peptic ulcer. Local production of gastrin may be increased without increased serum gastrin (hypergastrinemia).

- **Multifocal atrophic gastritis:** With advancing time, the antral gastritis may progress to involve the more proximal stomach (i.e. body and fundus) producing multifocal atrophic gastritis. This is characterized by patchy mucosal atrophy, reduced parietal cell mass and acid secretion, intestinal metaplasia, and has an increased risk of developing adenocarcinoma of stomach. Thus, there is an inverse relationship between duodenal ulcer and gastric adenocarcinoma. *H. pylori* do not invade and they are not found in the absence of foveolar cells or in association with intestinal-type epithelium. Thus, wherever there is intestinal metaplasia, *H. pylori* are not found.

H. pylori: Gastric antrum is the most common site of colonization.

Mechanism of Action

- **Flagella:** It makes them motile, allows it to burrow and live beneath the mucus layer above the epithelial surface.
- **Urease:** Produced by *H. pylori* releases ammonia (strong alkali) from endogenous urea, raises the local gastric pH and enhances bacterial survival. Raised local gastric pH acts on the antral G cells and releases gastrin → hypergastrinemia result in **hypersecretion of gastric acid.**
- **Adhesins:** It helps *H. pylori* to bind to the surface of foveolar cells of gastric epithelium.
- **Cytotoxins:** Products of two genes, namely cytotoxin-associated gene A (*cagA*) and vacuolating agent (*vacA*) gene involved in the progression of the disease and cause gastritis, peptic ulceration and cancer.

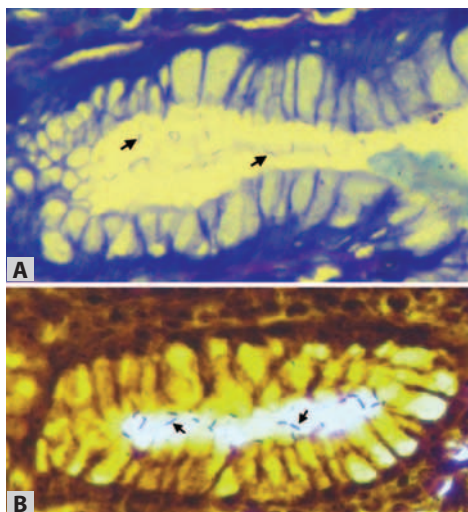
H. pylori:

1. Produces urease
2. Noninvasive
3. Colonizes in the mucosal layer
4. Causes intestinal metaplasia—precursor of gastric cancer.

Host factors: Play role in the outcome of *H. pylori* infection.

- **Increased expression of pro-inflammatory cytokines:** Normally, *H. pylori* do not invade the cells/tissues. It causes increased production of pro-inflammatory cytokines [e.g. interleukin-1 β (IL-1 β), tumor necrosis factor (TNF)] by the mucosal epithelial cells.
- **Decreased expression of the anti-inflammatory cytokines:** E.g. interleukin-10 (IL-10).

The above mechanism activates neutrophils and macrophages (inflammatory response in the gastric



Figs 18.4A and B: Photomicrograph shows *H. pylori* (arrows) in the lumen of gastric glands: (A) Giemsa stain; (B) Warthin-Starry stain

mucosa) and impairs mucosal defense→pangastritis, atrophy and gastric cancer.

Thus, development and course of *H. pylori* gastritis depends on interplay between gastroduodenal mucosal defenses, inflammatory responses and bacterial virulence factors.

Mechanism of action of *H. pylori*:

- Flagella
- Urease
- Its products (adhesive molecules, enzymes and cytotoxins).

H. pylori induced gastritis: Due to the imbalance between gastroduodenal mucosal defenses and damaging forces.

Humans are the only known host for *H. pylori*.

Microscopy

- Presence of **intraepithelial neutrophils** and **subepithelial (superficial lamina propria) plasma cells** are **characteristics of *H. pylori* gastritis**. Neutrophils may accumulate in the lumen of gastric pits to produce pit abscesses.
- In **long-standing cases, gastritis may extend proximally to involve the body and fundus. Then the mucosa can become atrophic with loss of parietal and chief cells. Microscopically, it is accompanied by lymphoid aggregates**, some with germinal centers. These lymphoid aggregates represent an induced form of **mucosa-associated lymphoid tissue** or MALT, which **can transform into lymphoma**.

H. pylori gastritis:

1. Intraepithelial neutrophils
2. Subepithelial (superficial lamina propria) plasma cells.

Demonstration of *H. pylori*

H. pylori: Giemsa stain, Warthin-starry stain.

- Initially, *H. pylori* gastritis is limited to gastric antrum and later may extend to involve the body and fundus.
- *H. pylori* are concentrated within the superficial mucus covering the epithelial cells in the surface and neck regions.
- *H. pylori* have an affinity for gastric epithelium and are generally not found in association with intestinal metaplasia or duodenal epithelium. Biopsy of the antral region is preferred for evaluation of *H. pylori* gastritis.
- **Special stains for *H. pylori***: Organisms can be easily demonstrated with special stains.
 - **Modified Giemsa stain and Diff-Quik** stain are popular, quick, cheap and easy to perform.

- **Silver stains: Warthin-Starry, Genta stain.**
- **Immunohistochemistry for *Helicobacter*.**

H. pylori: Increased risk of—

- Gastric adenocarcinoma
- Gastric MALT lymphoma.

Diagnostic Tests for *H. pylori*

H. pylori: Biochemical reactions positive for—

- Catalase
- Oxidase
- Urease.

- Demonstration of *H. pylori* in tissue (**remains the gold standard for detection of *H. pylori***)
- Serologic test for antibodies to *H. pylori*
- Fecal bacterial detection
- Urea breath test based on the generation of ammonia by the bacterial urease

H. pylori: Rapid urease test on gastric mucosal biopsy is 100% specific test.

- Gastric biopsy specimens: Histological examination (refer special stains mentioned above), analysis by the rapid urease test, bacterial culture or bacterial DNA detection by PCR.

Peptic ulcer disease is a complication of chronic *H. pylori* gastritis.

H. pylori: Grows well at 37°C in microaerophilic conditions. Culture media includes—

- Skirrow's medium
- Chocolate medium.

PEPTIC ULCER DISEASE

Q. Define peptic ulcer.

Q. Discuss the pathogenesis of peptic ulcer.

Definition: Peptic ulcer is defined as a **chronic mucosal ulceration/defect that penetrates the muscularis mucosae**. It usually **affects the duodenum (duodenal ulcer) or stomach (gastric ulcer)**.

Peptic ulcer disease (PUD) is one of the complications of chronic gastritis. It is **most often associated with colonization with *H. pylori*** and *H. pylori*-induced chronic gastritis (with hyperchlorhydria), NSAIDs, or cigarette smoking.

Peptic ulcer: Mucosal defect that is at least 0.5 cm in diameter penetrates the muscularis mucosae.

Normal Process in the Stomach

The gastric lumen pH is about 1 which is several times more than the pH of blood. Though it is needed for the digestion, sometimes it can damage the gastric mucosa. There are several mechanisms which protect the gastric mucosa.

Two opposing sets of forces keep stomach in a normal state: A. damaging forces and B. Defensive forces (Fig. 18.5).

Damaging Forces

These forces are capable of inducing mucosal injury and consists of two gastric secretory products: 1) hydrochloric acid and 2) pepsinogen.

Gastric Acidity

Hydrochloric acid plays **main role in digestion** but it also **can damage the gastric mucosa**.

- Enzyme H^+/K^+ ATPase is responsible for producing the large concentration of H^+ . Acid is secreted by the parietal cell located in the oxyntic gland.
- Parietal cell expresses receptors for many stimulants of acid secretion, including histamine (H_2), gastrin (cholecystokinin B/gastrin receptor), and acetylcholine (muscarinic receptor, M_3).

Peptic Enzymes

They **can also damage** the gastric mucosa.

- Pepsinogen, which is an inactive precursor of pepsin, is synthesized and secreted by the chief cell, found mainly in the gastric fundus.
- The acid environment within the stomach leads to conversion of pepsinogen to pepsin and provides the low pH (<2.0) required for activity of pepsin.
- Pepsin activity decreases as the pH increase; it is markedly decreased at a pH of 4 and inactivated at a pH of 7 and above. Many of the substances, which stimulate acid secretion, also stimulate pepsinogen release.

Defensive Forces

These are a three-level barrier composed of: 1) Pre-epithelial, 2) epithelial and 3) subepithelial elements.

Pre-epithelial Barrier

It is a mucus-bicarbonate layer of the stomach. It is formed by several factors produced by surface epithelial cells, such as: a) Production of mucus, b) bicarbonate secretion, c) epithelial cell ionic transporters that maintain intracellular pH and d) intracellular tight junctions.

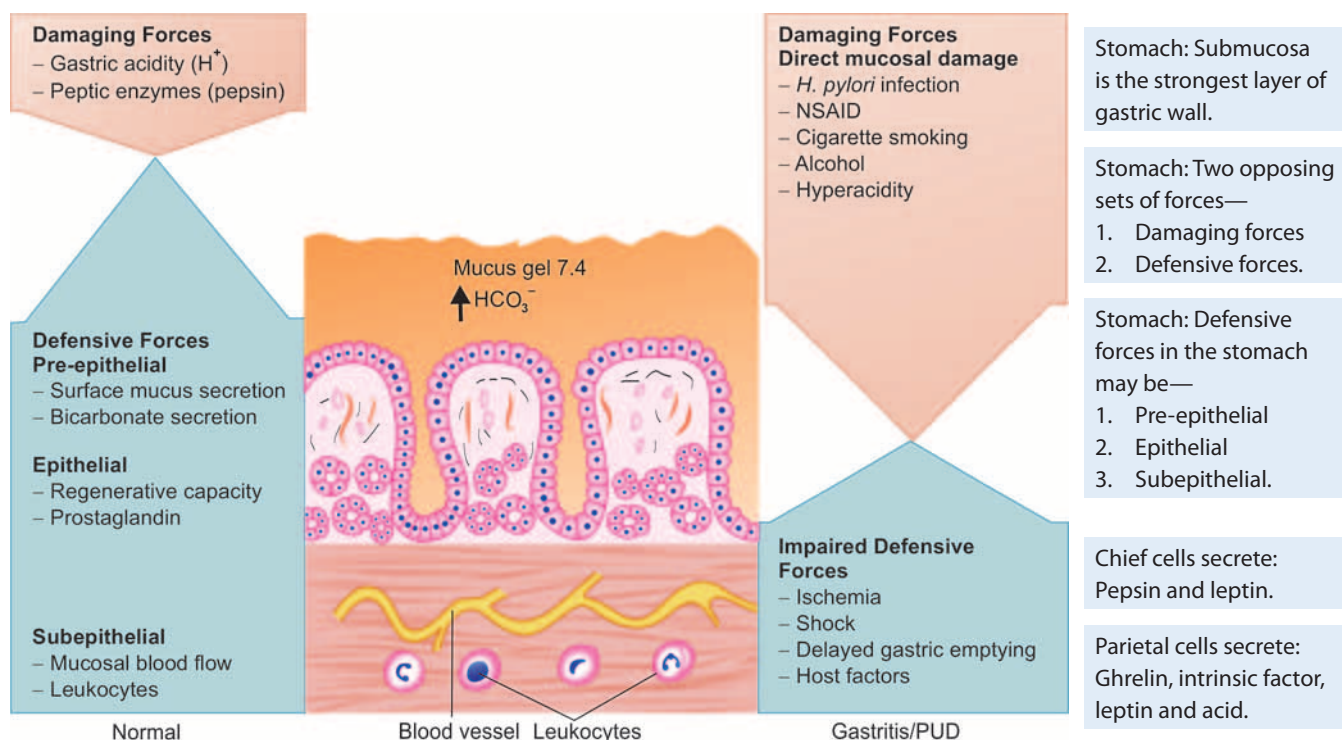


Fig. 18.5: Components involved in mucosal defense and repair in normal (left side) and in acute or chronic gastritis (right side). Gastric mucus barrier consists of viscid mucus (forms an unstirred layer between the epithelium and the gastric lumen) and bicarbonates

- **Surface mucus secretion:** Mucin is secreted by surface foveolar cells. Actions of mucus are:
 - Mucus layer **promotes formation of an “unstirred” protective layer** of fluid on the mucosa.
 - **Prevents the direct contact of large food particles to the epithelium.**
 - Impedes the diffusion of ions and molecules such as pepsin.
- b. **Bicarbonate secretion:** Surface epithelial cells secrete bicarbonate into the mucus → bicarbonate diffuses into the unstirred mucus → buffer the hydrogen ions entering from the luminal aspect. It results in a pH gradient, ranging from 1 or 2 at the gastric luminal surface, and reaching to a neutrality of 6 to 7 along the epithelial cell surface.

Epithelial Barrier

It consists of surface epithelial cells that acts through a) restitution of damaged gastric epithelial cells, b) epithelial regeneration, c) secretion of prostaglandins and d) production of mucus (see above).

- **Restitution:** It is the process of restoration of a damaged region by the gastric epithelial cells and requires continuous blood flow and an alkaline pH in the surrounding environment.
- **Epithelial regeneration:** It is regulated by prostaglandins and growth factors such as EGF and TGF- α .
- **Secretion of prostaglandins:** Gastric mucosa secretes prostaglandin which plays a main role in gastric epithelial defense/repair. **Prostaglandins maintain mucosal blood flow and epithelial cell restitution.**

Prostaglandins protect gastric mucosa by:

1. Increasing secretion of bicarbonate
2. Increasing vascular perfusion
3. Inhibition of acid secretion
4. Promoting synthesis of mucin.

Subepithelial Barrier

- **Rich gastric mucosal blood flow:** It provides (1) bicarbonate (HCO_3^-), which neutralizes the acid generated by parietal cells, (2) an adequate supply of nutrients and oxygen, and (3) removes toxic metabolic by-products.

Pathogenesis of PUD/Acute or Chronic Gastritis

PUD usually develops on a background of chronic gastritis.

Q. Discuss the role of *H. pylori* in gastric ulcer.

The imbalances between mucosal defenses and damaging forces cause chronic gastritis and also PUD.

PUD can occur due to direct mucosal injury or disruption of any of protective mechanisms.

Peptic ulcer disease: *H. pylori* infection and use of NSAID are the main causes.

Risk factors for peptic ulcer disease are listed in Box 18.2.

BOX 18.2: Risk factors for peptic ulcer disease

H. pylori infection: Risk factors

- Cigarette smoking
- NSAIDs (non-steroidal anti-inflammatory drugs)
- Chronic obstructive pulmonary disease (COPD)
- Alcoholic cirrhosis
- Psychological stress
- Zollinger-Ellison Syndrome

Direct Mucosal Injury/Increased Damage

1. ***H. pylori* infection** (refer above) is one of the **most important, common, primary cause** of PUD. *H. pylori* is detected from the gastric antrum in almost all patients with duodenal ulcers. It is associated with increased secretion of gastric acid and reduced duodenal secretion of bicarbonate.

H. pylori infection is associated with:

~90% of duodenal ulcers

~65% of gastric ulcers.

2. **Nonsteroidal anti-inflammatory drugs (NSAIDs) and aspirin:** It causes 1) **direct chemical irritation of mucosa**, 2) **suppresses mucosal prostaglandin synthesis** and 3) **reduces the bicarbonate secretion.**

NSAID and aspirin causes:

1. Direct chemical irritation of mucosa
2. Suppresses prostaglandin synthesis by mucosa
3. Reduces the bicarbonate secretion.

3. **Cigarette smoking:** **Impairs blood flow to the mucosa** and healing of mucosal damage.
4. **Alcohol**, radiation therapy and chemotherapy: They cause direct injury to mucosal cells.
5. **Ingestion of chemicals:** These include acids or bases and cause direct injury.
6. **Gastric hyperacidity:** The causes of hyperacidity include *H. pylori* infection, parietal cell hyperplasia and Zollinger-Ellison syndrome (refer page 486).
7. **Others**
 - **High-dose corticosteroids:** They suppress prostaglandin synthesis and impair healing.
 - Psychological stress.
 - Duodenal gastric reflux.

Chronic disorders associated with PUD:

1. Cirrhosis
2. Chronic pulmonary disease
3. Chronic renal failure
4. Alpha 1-antitrypsin deficiency
5. Systemic mastocytosis
6. Nephrolithiasis.

Impaired Defense

- **Ischemia:** Decreased oxygen delivery (e.g. at high altitudes and shock)
- **Shock**
- **Delayed gastric emptying**
- **Host factors:** Reduced mucin synthesis in the elderly → increased susceptibility to gastritis.

MORPHOLOGY

Q. Write short note on morphology of peptic ulcer.

Gross (See Fig. 18.5)

Sites of peptic ulcer:

1. Duodenum
 2. Stomach
 3. Esophagus
 4. Jejunum
 5. Gastric mucosa in Meckel diverticulum.
- **Sites of peptic ulcer:** They can develop in any portion of the GI tract exposed to acidic gastric juices.
 - **Duodenum:** More common in the first portion of the duodenum (anterior or posterior wall) within a few centimeters of the pyloric valve **than in the stomach**.
 - **Stomach:** Lesser curvature near the junction (transitional zone) of the body and antrum:
 - ◆ Proximal ulcers: Located in the body of the stomach.
 - ◆ Distal ulcers: Located in the antrum and angulus of the stomach.
 - **Gastroesophageal junction of esophagus.**
 - **Anastomotic site:** It can develop at the anastomotic site in patients who have undergone a distal gastric resection. Occur at margins of the gastroduodenal anastomosis/gastrojejunostomy (anastomotic ulcer).
 - **Multiple ulcers:** In the duodenum, stomach, and/or jejunum in Zollinger-Ellison syndrome.
 - **At metaplastic or heterotopic gastric mucosa:** E.g. Meckel diverticulum within an ileum having ectopic gastric mucosa.

Peptic ulcer: Most common sites—

- Duodenal ulcer: First part of duodenum
- Gastric ulcer: Lesser curvature along the incisura angularis.

- **Number:** **Solitary** in more than 80% of patients, but may be more than one.
- **Size:** Lesions less than 0.3 cm in diameter are shallow and those larger than 0.6 cm are likely to be deeper ulcers.
- **Shape:** **Round to oval, sharply punched-out defect.**
- **Margin:** Usually **in level with the surrounding mucosa**. The gastric mucosal folds can be traced up to the margins of ulcer and the radiating folds of mucosa from ulcer (see Fig. 18.5) appear like a spoke wheel. In contrast, **heaped-up margins are more characteristic of gastric cancers**.
- **Depth:** Varies.
- **Base:** It is **smooth and clean** as a result of peptic digestion of exudate.

Gastric ulcer: Size and location cannot differentiate benign and malignant ulcers.

Kissing ulcers: Peptic ulcers occurring at both a posterior and anterior wall of the duodenum.

Microscopy (Figs 18.6 and 18.7)

- Gastric and duodenal ulcers are microscopically similar. From the lumen outward **four layers** can be identified and are known as **Askanazy zones**.
 - **Necrotic zone:** It is the most superficial zone.
 - **Superficial exudative zone:** It consists of **fibrinopurulent exudates with predominantly neutrophilic inflammatory infiltrate**.
 - **Granulation tissue zone:** It consists of granulation tissue infiltrated with mononuclear leukocytes.
 - **Zone of cicatrization:** It consists of **fibrous tissue or collagenous scar** which forms the base of the ulcer and may show chronic inflammatory cells.

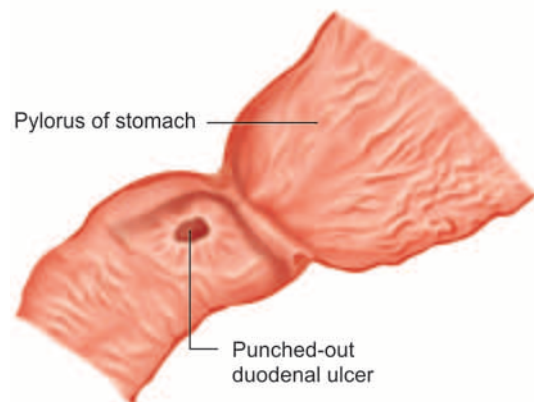
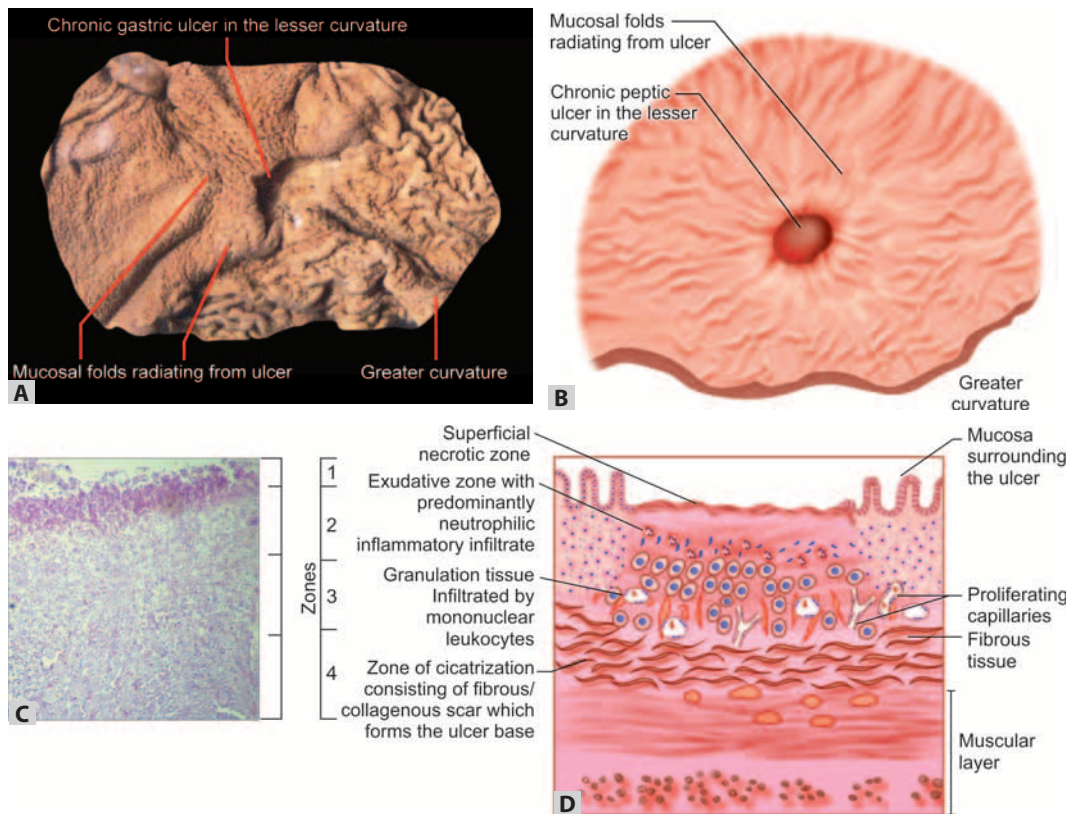


Fig. 18.6: Duodenal ulcer in the first part of duodenum showing characteristic sharp demarcation from the surrounding mucosa



Malignant transformation of peptic ulcers is very rare.

Microscopy of peptic ulcer: From the lumen outward four layers can be identified and are known as Askanazy zones.

PUD: Duodenal > gastric.

Autoimmune gastritis involves fundus and body of stomach.

Figs 18.7A to D: Peptic ulcer of stomach (A. Specimen; B. Diagrammatic) showing characteristic sharp demarcation from the surrounding mucosa. Microscopic features of a peptic ulcer (C. Photomicrograph and D. Diagrammatic) showing four layers of chronic gastric ulcer

Clinical Features

Peptic ulcer: Once a peptic ulcer, always a peptic ulcer patient.

- Peptic ulcers are **chronic, recurring lesions with more morbidity than mortality**.
- **Age:** Young adults but are most often diagnosed in middle-aged to older adults.
- **Periodicity:** After a period of weeks to months of active disease, healing may occur with or without treatment.
- **Pain:** Epigastric burning or aching pain exacerbated by fasting and improved with alkali or food. It usually develops 1–3 hours after meals during the day and is worse during night between 11 PM and 2 AM.
- **Other symptoms:** These include nausea, vomiting, bloating, belching and significant weight loss.

Severe bleeding may cause “coffee ground” vomitus or melena and may be life-threatening.

- **Perforation:** Develops in ~ 5% of patients and is the **most common complication of gastric ulcer**.
- **Pyloric obstruction (gastric outlet obstruction):** It is associated with ulcers in the pyloric region and occurs in ~ 10% of ulcer patients. It is secondary to either edema or scarring.
- **Rarely malignant transformation:** The dictum is that ‘cancers ulcerate but ulcers rarely cancerate’.

PUD:

Peptic ulcers in the duodenum never become malignant.

Small percentage of gastric ulcers may undergo malignant transformation.

- **Development of combined ulcers:** In the stomach and duodenum in the same patient.

Complications of duodenal ulcers:

1. Bleeding—most common
2. Perforation: Most common from anterior aspect of first part of duodenum.

Complications of Gastric Ulcers

Q. Complication of gastric ulcer.

- **Bleeding:** Most common complication of peptic ulcer. Chronic blood loss may lead to iron deficiency anemia.

TABLE 18.2: Differences between gastric and duodenal peptic ulcer

Features	Gastric ulcer	Duodenal ulcer
Commonest site	Along the lesser curvature	First part of duodenum
Incidence	Less common	More common
Age	Beyond 6th decade, M>F	Between 25 and 50 years, M>F
Association with <i>H. pylori</i> infection	Less common	Strong association
Acid level	Usually normal	High
Clinical features		
Relationship of pain to antacids	Relief of pain not consistent	Prompt relief of pain
Relationship of pain to food	Aggravates the pain	Relieves the pain
Night pain	Not observed	Common
Heart burn	Not common	Common
Hematemesis/melena	Hematemesis more common	Melena more common
Vomiting	Common	No vomiting
Weight loss	Present	Absent
Complication	Rarely undergo malignant change	No malignant change

Gastroduodenal artery is the source of bleeding in duodenal ulcer.

Left gastric artery is the source of bleeding in gastric ulcer.

Urea breath test is used to ensure the efficacy of the treatment for peptic ulcer disease caused by *H. pylori*.

Differences between gastric and duodenal peptic ulcer are given in Table 18.2.

ZOLLINGER-ELLISON SYNDROME (ZES)

Q. Write short note on Zollinger-Ellison syndrome.

Triad: (1) Hypergastrinemia caused by gastrin-secreting tumors (gastrinomas), (2) increased acid production, and (3) severe peptic ulcer disease.

- **Hypergastrinemia:** **Gastrinomas** are commonly seen in the small intestine or pancreas → increased gastrin secretion → leads to massive secretion of **acid and pepsin**.

ZES: Fasting serum gastrin level greater than 1000 pg/mL is diagnostic.

- **Increased acid secretion** → inactivation of pancreatic enzymes and bile salts → leading to malabsorption and diarrhea. Stomach shows **increase in the number of parietal cells**.
- **Multiple and recurrent peptic ulcers** in the duodenum, stomach and/or jejunum.

ZES: Caused by gastrin-secreting tumors (gastrinomas) → massive acid production → hyperacidity → single or multiple peptic ulcers (stomach, duodenum, and even jejunum).

GASTRIC ADENOCARCINOMA

Adenocarcinoma is the most **common malignancy of the stomach**. It comprises ~ 90% of all gastric cancers.

Epidemiology

Antrum: Most common site for—

- Gastric carcinoma
- Gastric lymphoma.

- More common in Japan and in males.
- Overall **decrease in the incidence** of gastric cancer is noted and may be due to:
 - Closely linked to decreases in *H. pylori* prevalence.
 - **Decreased consumption of dietary carcinogens**, such as (1) N-nitroso compounds and benzo[a]pyrene, (2) reduced use of salt and smoking food, (3) decreased use of preservatives due to easy availability of food refrigeration.

Fundus: Most common site for—

- Gastric carcinoma in pernicious anemia
- Diffuse type of gastric carcinoma.

- **Intake of green, leafy vegetables and citrus fruits** that contain antioxidants such as vitamin C, vitamin E and beta-carotene.

Though overall there is reduction of cancer of stomach, the cancer of the cardia region is on the rise. This may be due to Barrett esophagus, increased incidence of chronic GERD and obesity.

Q. Mention the classification of gastric carcinoma.

WHO classification of gastric tumors are listed in Table 18.3.

TABLE 18.3: WHO histologic classification of gastric tumors

Epithelial tumors	Mesenchymal tumors
Premalignant lesions: Intraepithelial neoplasia, adenoma	• Leiomyoma
Adenocarcinoma	• Schwannoma
• Papillary adenocarcinoma	• Granular cell tumor
• Tubular adenocarcinoma	• Leiomyosarcoma
• Mucinous adenocarcinoma	• Gastrointestinal stromal tumor (GIST)
• Signet-ring cell carcinoma	• Kaposi sarcoma
• Undifferentiated carcinoma	• Others
Adenosquamous carcinoma	<i>Malignant lymphoma</i>
Neuroendocrine neoplasms	
• Carcinoid tumor	

Classification is also done according to location, gross and microscopic appearance (refer page 488-489).

Etiology and Pathogenesis

Gastric cancer is a multifactorial disease.

Risk Factors (Box 18.3)

Q. Discuss the etiology and pathogenesis of gastric carcinoma.

H. pylori increases the risk of gastric carcinoma by 5 to 6 fold.

Helicobacter pylori infection: It increases the risk by five- to six-fold. However, majority of individuals infected with *H. pylori* does not develop cancer. Chronic *H. pylori* infection → mucosal inflammation → decreases secretion of acid (hypochlorhydria) and pepsin → favors bacterial growth and continuation of chronic inflammation → progresses to mucosal atrophy → intestinal metaplasia → dysplasia → carcinoma.

Gastric carcinoma is associated with blood group: A.

Risk factors for carcinoma stomach: Environmental, host and genetic.

Carcinoma of stomach: Alcohol is **NOT** a risk factor.

Decreased risk of carcinoma stomach with:

1. Aspirin
2. Diet rich in fresh fruit and vegetables
3. Vitamin A and C
4. Calcium, selenium, zinc and iron.

BOX 18.3: Risk factors of gastric carcinoma

A. Environmental factors

1. *H. pylori* infection (intestinal-type carcinoma and Epstein-Barr virus infection)
2. Dietary/nutritional: Nitrites derived from nitrates (water, preserved food), smoked foods (due to benzpyrene) and excess of salt (salted pickled vegetables, chili peppers), and deficiency of fresh fruit, vegetables, vitamins A and C, refrigeration
3. Low socioeconomic status: Low fat or protein consumption, high complex carbohydrate consumption
4. Rubber and coal workers

B. Host factors/predisposing conditions

1. Chronic gastritis (especially atrophic gastritis), pernicious anemia: Hypochlorhydria: Favors colonization with *H. pylori*. Intestinal metaplasia is a precursor lesion.
2. Partial gastrectomy: Favors bile reflux, alkaline intestinal fluid
3. Gastric dysplasia and adenomas more than 2 cm
4. Ménétrier's disease (hypertrophic gastropathy)
5. Barrett esophagus

C. Genetic factors

- Blood group A, family history of gastric cancer, hereditary nonpolyposis colon cancer (HNPCC) syndrome, familial gastric carcinoma syndrome (E-cadherin mutation), Li-Fraumeni syndrome.

Pathogenesis

Majority of gastric cancers have no hereditary basis. However, mutations detected in familial gastric cancer have helped in understanding the mechanisms of carcinogenesis in sporadic cases.

Familial Gastric Cancer

- Germline loss-of-function mutations in the tumor suppressor gene *CDH1* strongly associated with familial gastric cancer. Gene *CDH1* encodes the cell adhesion protein E-cadherin.

Sporadic Gastric Cancer

- **Sporadic diffuse gastric cancers** show loss of E-cadherin and is the key step in the development of diffuse gastric cancer. This may be by the following mechanisms:
 - **Loss-of-function mutations in the tumor suppressor gene *CDH1*** is also observed in about 50% of sporadic diffuse gastric tumors.
 - **Hypermethylation and silencing of the *CDH1* promoter** which decreases the E-cadherin expression.
 - **BRCA2 mutations:** Increased risk of diffuse gastric cancer.

CDH1 mutations are also common in sporadic and familial lobular carcinoma of the breast. These breast tumors similar to diffuse gastric carcinoma infiltrate as single cells.

- **Sporadic intestinal-type gastric cancers.**
 - **Mutations causing increased signaling via the Wnt pathway:** This may be due to loss of- function mutations in the adenomatous polyposis coli (APC) tumor suppressor gene and gain-of-function mutations in the gene encoding β -catenin.
- **Mutation of *TP53*** in majority of sporadic gastric cancers of both diffuse and intestinal type.
- **Loss-of-function mutations in other genes:** These include other tumor suppressor genes, genes involved in regulation of apoptosis (*BAX*), and cell cycle control (*CDKN2A*).

MORPHOLOGY

Q. Write short note on morphology of gastric carcinoma.

Site

- Pylorus and antrum (50–60%)
- Cardia (25%)
- Body and fundus (15–25%).

Lesser curvature is involved more often (in ~ 40%) than the greater curvature (~12%). Most favored site is the lesser curvature of the **antropyloric region**.

Carcinoma stomach: Lesser curvature of pyloric and antral region are the most common sites.

Classification of Gastric Carcinoma

Classification: Based on—

1. Depth of invasion
2. Macroscopic growth pattern
3. Histologic subtype.

Q. Classify gastric carcinoma.

There are several classifications of carcinoma of stomach.

- WHO classification of gastric tumors:** It is **based on the histological features** and is presented in Table 18.3.
- Based on depth of invasion:** Important morphological feature that decides the clinical outcome of gastric cancer is depth of invasion. According to this, gastric cancer can be divided into early gastric cancer and advanced gastric cancer.
 1. **Early gastric carcinoma** (Fig. 18.8): It is defined as an invasive cancer that is limited to the mucosa and submucosa, with or without perigastric lymph node metastases. It is not synonymous with carcinoma in situ (which is confined to the surface epithelial layer). Early gastric cancer is a **pathologic diagnosis** based on depth of invasion and is associated with better prognosis.

An ulcerative lesion on the greater curvature is more likely to be malignant.

2. **Advanced gastric carcinoma:** It is a neoplasm that has **extended below the submucosa into the muscular wall**. All cancers may begin as “early” lesions, which develop into “advanced” lesions. It is associated with poor prognosis.
- C. Based on the macroscopic growth pattern** (Fig. 18.8): **Three patterns** are observed and are used for both early and advanced gastric cancers.
1. **Type I (exophytic/polypoid/fungating):** It is a solid tumor **which projects/protrudes into the lumen** as a polypoid or nodular mass.
 2. **Type II (flat or depressed):** It is a **superficial, flat lesion** with no obvious tumor mass within the mucosa and may be slightly elevated or depressed. Three patterns are 1) elevated (type IIa) 2), flat (type IIb), and 3) depressed (type IIc).
 3. **Type III (excavated):** It is characterized by a **shallow or deeply erosive crater** in the wall of the stomach. An excavated malignant ulcer does not ordinarily occur alone but rather represents ulceration of type I or type II tumors.
 - ♦ They may closely **resemble chronic peptic ulcers**. The **mucosa surrounding the ulcers appears firm, raised and nodular**.
 - ♦ Characteristically, the **margins** of the ulcer are **heaped-up, beaded**, shaggy and irregular and the **base is ragged and necrotic**. This is in contrast to that of the benign peptic ulcer, which shows punched-out margins and a smooth base.
- D. Lauren classification depending on the histologic subtype:** According to this, there are two important types: Intestinal type and diffuse type.

Carcinoma of stomach: Intestinal type and diffuse type.

1. Intestinal type:

- ♦ **Gross:** It forms **polypoid bulky tumors** (Fig. 18.9A) or may be **ulcerated** (Fig. 18.9B).
- ♦ **Microscopy** (Figs 18.10A and B): It consists of cohesive tumor cells that **form gland-like tubular structures**, resembling adenocarcinoma of colon. It probably **arises in areas of intestinal metaplasia**. The tumor cells show **apical mucin vacuoles**, and mucin may be present in the lumen of the glands

2. Diffuse or infiltrating gastric adenocarcinoma:

- ♦ **Gross:** Diffuse gastric cancer **infiltrates deeply into the stomach without forming obvious mass lesions**. It involves broad region or entire stomach with local rigidity of the wall. They elicit a **desmoplastic reaction** → produces diffuse **flattening of rugal folds** in the mucosa and rigid, widespread **thickening of the wall**. If the entire stomach is involved, it may become nondistensible and lumen is narrowed → producing **leather bottle appearance** termed **linitis plastica** (Fig. 18.9C).

- ♦ **Microscopy:** It is composed of **discohesive** (cohesion is absent because of loss of E-cadherin) **tumor cells** which **do not form glands**. The tumor cells infiltrate and thicken the stomach wall without forming a discrete mass.
 - ◊ The **tumor cells contain abundant mucin** which expand the cytoplasm and push the nucleus to the peripheryà creating **signet-ring cell** (Figs 18.11A and B) appearance.
 - ◊ If the signet-ring cells constitute **more than 50%** of the tumor, it is classified as **signet-ring cell carcinoma**.

Spread of Carcinoma Stomach

Q. Write short note on modes of spread of gastric cancer (carcinoma stomach).

1. **Local/direct spread:** It spreads into the muscularis and serosa of the stomach. It can invade into the duodenum, pancreas, liver, colon and retroperitoneum.
2. **Lymphatic spread:** It spreads via the submucosal and subserosal lymphatics to both regional and distant

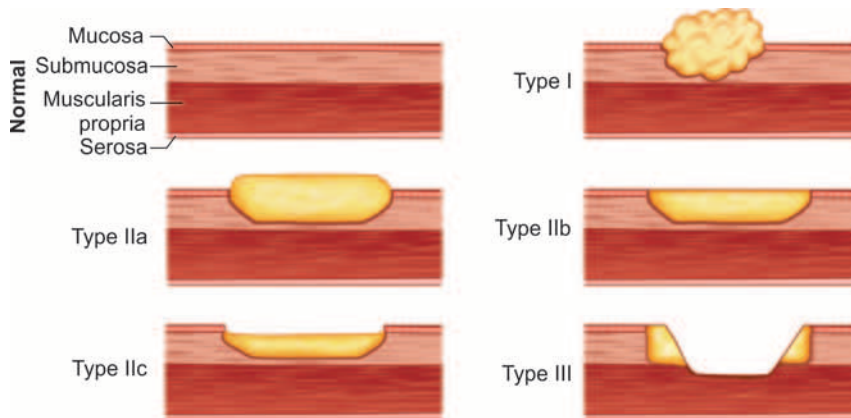
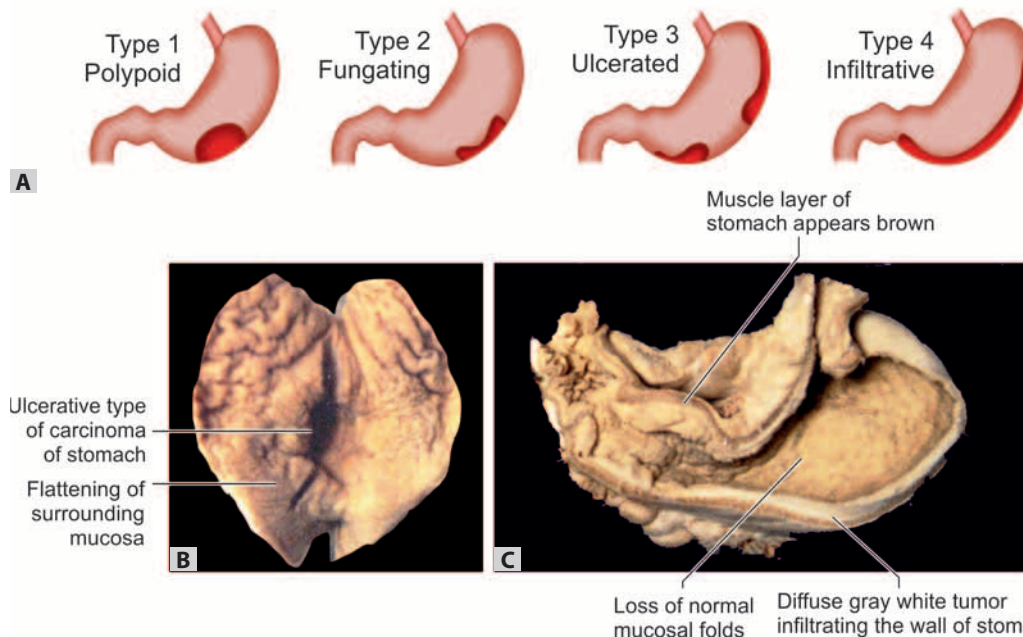


Fig. 18.8: Japanese classification of early gastric cancer

Early gastric carcinoma: Does not refer to the duration of the disease, its size, presence of symptoms and absence of metastases or curability.

Early gastric carcinoma: Tumor confined to the mucosa and submucosa irrespective of lymph node status.



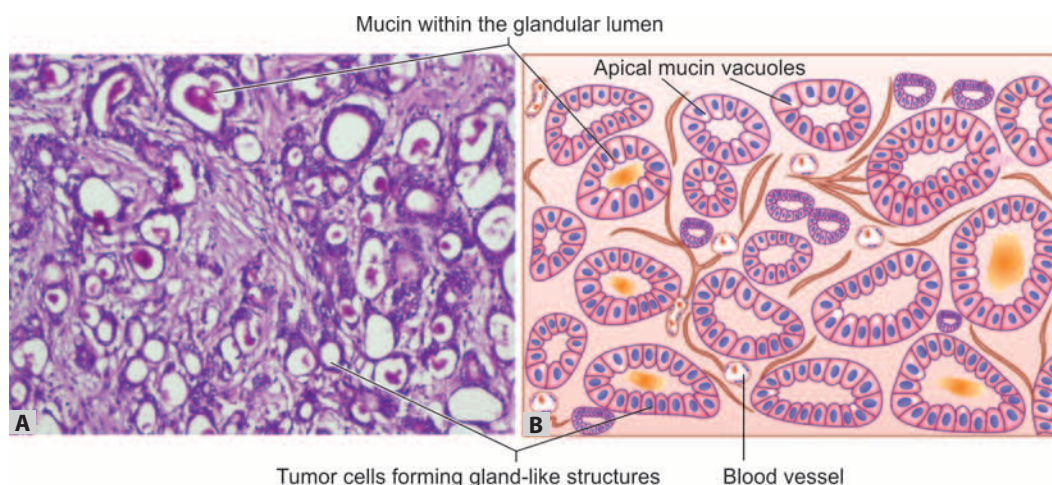
Sister Mary Joseph nodule/ node/sign:

Palpable nodule bulging into the umbilicus due to metastasis of cancer in the pelvis or abdomen.

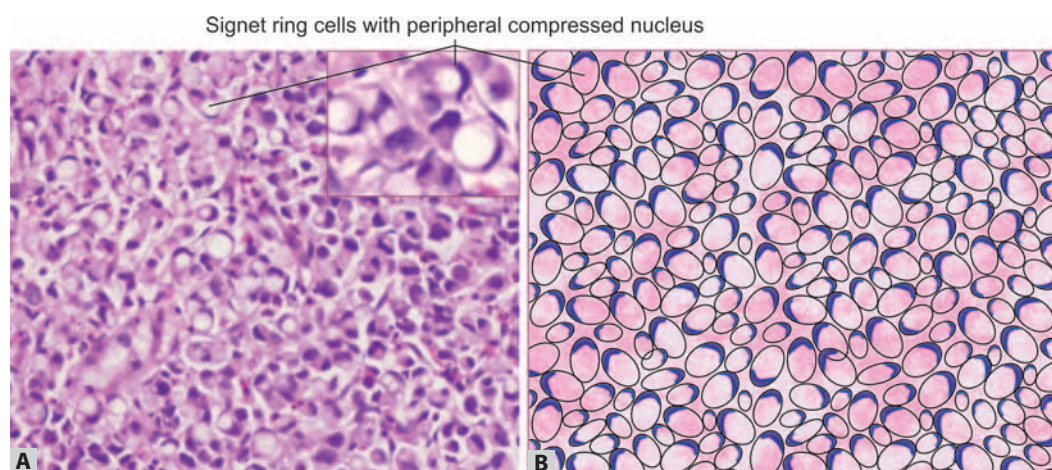
Diffuse or infiltrating gastric cancer (linitis plastica): **Not** associated with *H. pylori*.

Linitis plastica (leather bottle stomach): Due to diffuse infiltrative growth that thickens the wall without forming a discrete mass.

Figs 18.9A to C: (A) Bormann classification of growth patterns of advanced gastric carcinoma. Gastrectomy specimen showing; (B) excavated ulcerative type of carcinoma (Bormann type 3); (C) diffuse type of carcinoma stomach with leather bottle appearance (Bormann type 4).



Figs 18.10A and B: Microscopic appearance of intestinal type of gastric carcinoma: (A) Photomicrograph; (B) Diagrammatic



Figs 18.11A and B: (A) Microscopic appearance of diffuse type of gastric carcinoma predominantly consisting of signet ring cells (signet ring carcinoma). Inset shows signet ring cells (upper right); (B) Diagrammatic appearance of signet ring carcinoma

Treatment of *H. pylori* reduces the risk of gastric cancer and lymphoma.

Signet-ring cell carcinoma: Signet-ring cells constitute more than 50% of the tumor.

lymph nodes. Pattern of lymph node involvement depends on the site of tumor (Table 18.4).

- Frequently metastasize to the **supraclavicular sentinel (Virchow) node** and may be the first clinical manifestation of an occult neoplasm (**Troisier's sign**).
 - Left axillary lymph node—Irish node

Gastric carcinoma: Virchow's left supraclavicular lymph node involvement due to metastasis (**Troisier's sign**).

- Metastasize to the **periumbilical lymph nodes**: This forms a subcutaneous nodule → called **Sister Mary Joseph nodule** (after the nurse who noted this lesion as a marker of metastatic carcinoma). This nodule represents metastasis from GI tract malignancies (in about 50% of cases) and includes carcinoma of

TABLE 18.4: Pattern of lymph node metastasis in gastric carcinoma

Site of tumor	Lymph nodes likely to be involved
Proximal stomach and GE junction	Mediastinal and pericardial lymph nodes
Body of stomach	Greater and lesser curvature nodes near the tumor
Tumors of distal stomach	Periduodenal, peripancreatic and porta hepatis nodes

stomach (most common), colon and pancreas. Other primary sites include carcinoma of ovary and uterus.

- Metastasis to the **ovaries** is called **Krukenberg tumor**.

3. **Blood spread:** It can spread via the portal vein into **liver**. Other sites are **lungs** and **bones**.

Sister Mary Joseph nodule/node: Associated with multiple peritoneal metastases. Prognosis poor.

Krukenberg tumor: Metastasis of signet-ring carcinoma to both ovaries. Primary may be in the stomach, breast, pancreas and gallbladder.

Clinical Features

- In high-risk areas, intestinal-type gastric cancer may arise from precursor lesions (e.g. flat dysplasia and adenomas).
- **Advanced cancer:** It presents with **early satiety, bloating, distension and vomiting**. The tumor frequently bleeds → **causing iron-deficiency anemia**. Tumor in the pyloric region may present with **gastric outlet obstruction**.
- **Troisier's sign:** Metastatic lymph nodes may be palpable in the left supraclavicular fossa (Virchow's node, Troisier's sign).
- **Trousseau's sign:** Non-metastatic effects may be in the form of **migrating thrombophlebitis** (Trousseau's sign) and **deep venous thrombosis**. These are due to the effects of the tumor on thrombotic and hemostatic mechanisms.

Prognosis: It depends on the **depth of invasion, the extent of nodal and distant metastasis**.

Differences between benign and malignant ulcers of stomach are listed in Table 18.5.

Gastric carcinoma:

- Abdominal pain > weight loss are the most common symptom
- Hematemesis is very rare
- Troisier's sign: Left supraclavicular lymph node metastasis.

GASTROINTESTINAL STROMAL TUMOR

GIST: Most common mesenchymal tumor of the abdomen.

- Gastrointestinal stromal tumor (GIST) is the **most common mesenchymal tumor of the abdomen**.
- **Most common location** of GIST is the **stomach**.

GIST: Most common location in the **stomach**.

Epidemiology

- **Sex:** Slightly more common in males.
- **Age:** Peak age for GIST in the stomach is about 60 years.

TABLE 18.5: Differences between benign and malignant ulcers of stomach

Q. List the differences between benign and malignant ulcers of stomach.

Characteristics	Benign ulcer	Malignant ulcer
Age	Usually in younger age	Older age
Duration of symptoms	Weeks to years	Weeks to months
Location/usual site	Lesser curvature of pylorus and antrum	Greater curvature of pylorus and antrum
Gross		
1. Size	Small	Large
2. Shape	Regular	Irregular
3. Surrounding mucosa	Mucosal rugae projects outwards (radiates) from the margins of the ulcer	Mucosal rugae stop far of the ulcer and shows flattening
4. Floor of the ulcer	Clean	Necrotic
5. Margins	Overhanging like spokes of wheel	Heaped up and everted
Acidity	Normal to low	May show achlorhydria

Infection with *H. pylori* is associated with distal intestinal type of gastric carcinoma and NOT with diffuse proximal carcinoma.

Gastric carcinoma: Tumor markers

1. CEA
2. CA-19.9
3. CA-125
4. CA72-4
5. Beta-hCG.

- **Carney triad:** Nonhereditary syndrome seen in young females. Triad consists of 1) gastric GIST, 2) paraganglioma and 3) pulmonary chondroma.
- **Increased incidence** of GIST in patients with **neurofibromatosis type 1**.

Cell of origin: GISTs arise from the **interstitial cells of Cajal**, that are located in the muscularis propria and serve as **pacemaker cells for gut peristalsis**.

Carney triad: Nonhereditary syndrome in young females.

1. Gastric GIST
2. Paraganglioma
3. Pulmonary chondroma.

GISTs arise from the **interstitial cells of Cajal (pacemaker cells for gut peristalsis)**.

Pathogenesis

- About **80%** of all GISTs have oncogenic, gain-of-function **mutations of the gene encoding the tyrosine kinase KIT** (receptor for stem cell factor).
- About 8% of GISTs have mutations that activate a related tyrosine kinase, platelet-derived growth factor receptor α (PDGFRA).
- Above mutations cause **constitutive activation of KIT or PDGFRA receptor tyrosine kinases** → produce intracellular signals → activate the RAS and PI3K/AKT pathways → **promote tumor cell proliferation and survival**.
- Few GISTs does not have neither mutated KIT nor PDGFRA show mutations in other genes involved in these pathways (e.g. NF1, BRAF, HRAS, or NRAS).

MORPHOLOGY

Gross

Size varies and may be quite large (upto 30 cm in diameter). Usually solitary, well-circumscribed, fleshy mass covered by intact or ulcerated mucosa, but can also project outward toward the serosa.

Microscopy

Consists of thin elongated **spindle cells** or epithelial-appearing (**epithelioid**) cells or **mixtures** of these two types of cells (Fig. 18.12A).

Diagnostic Markers

GISTs and Cajal cells express **KIT** (also known as **CD117**) and CD34. Most **useful diagnostic marker is KIT** (Fig. 18.12B), and immunohistochemically detected in 95% of gastric GISTs.

GIST: Most useful diagnostic marker **KIT (CD117)** in 95% of cases.

Clinical Features

- Symptoms may be due to mass effects.
- Mucosal ulceration can cause blood loss.
- **Investigations:** Best diagnosed with CT scan and PET scan.

Prognosis

Correlates with:

1. **Tumor size:** Recurrence or metastasis is rare when size is less than 5 cm.
2. **Mitotic index:** Mitotically active tumors larger than 10 cm commonly metastasize or recur.
3. **Location:** Gastric GISTs is less aggressive than those of the small intestine.

Imatinib, a tyrosine kinase inhibitor that inhibits KIT and PDGFRA often useful in patients with unresectable, recurrent, or metastatic tumor.

MECKEL DIVERTICULUM

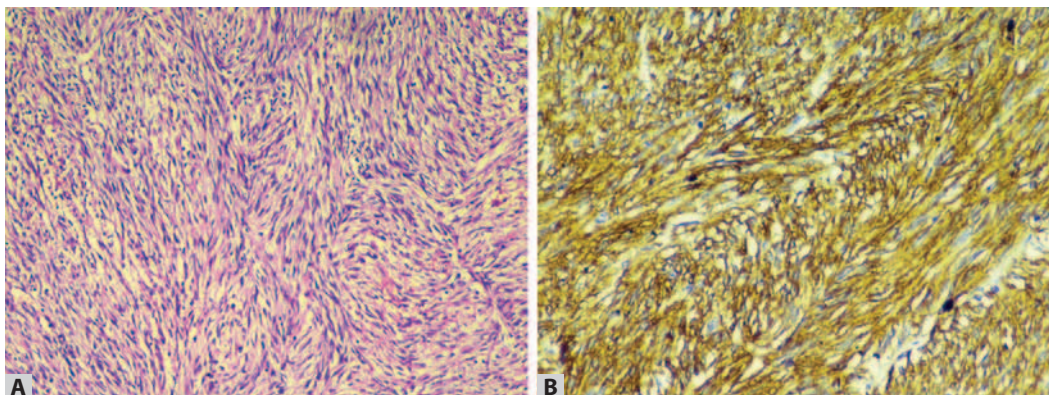
Q. Write short note on Meckel diverticulum.

Meckel diverticulum is the most common type of true diverticula of GI tract and occurs in the ileum.

Meckel diverticulitis: Symptoms mimic acute appendicitis.

Rule of 2s: It is used to remember characteristics of Meckel diverticulae.

- Occur in about **2%** of the **population**.
- Generally present within **2 feet** of the **ileocecal valve**.
- ~ **2 inches long** (varies from 2 to 15 cm).



Figs 18.12A and B: (A) Gastrointestinal stromal tumor of stomach showing bundles and fascicles of spindle-shaped tumor cells; (B) Tumor cells show KIT (CD117) positivity

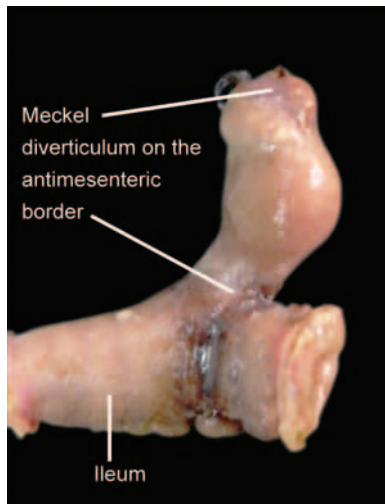


Fig. 18.13: Meckel diverticulum on the antimesenteric border

- Two times common in **males** than in females.
- Most often **symptomatic** by age 2.

MORPHOLOGY

- **Single** and always lies on the **antimesenteric** side of the bowel (Fig. 18.13).
- **True diverticulum:** The wall consists of mucosa, submucosa and muscularis propria and has its own blood supply.
- Lining of **epithelium** resembles that of **normal small intestine**, but **ectopic pancreatic or gastric tissue** is common. **Heterotopic gastric mucosa** may lead to **peptic ulceration** of adjacent small intestinal mucosa and present with **bleeding, or perforation**.

Complications

Meckel diverticulum: Bleeding is the most common complication.

- **Severe hemorrhage:** Develops if the diverticulum contains acid-secreting gastric epithelium with peptic ulceration → maroon colored painless bleeding per rectum.
- **Meckel's diverticulitis:** Develops secondary to peptic ulcerations or obstruction of the orifice of the diverticula and symptoms appear similar to acute appendicitis.
- **Intestinal obstruction:** Results from intussusception, volvulus, adhesions or the presence of a tumor or ectopic or enteroliths.

Submucosa: Strongest layer of the gut and surgically, it provides strength to intestinal anastomosis.

TYPHOID FEVER

- Typhoid fever (enteric) is an **acute systemic disease** caused by infection with *Salmonella typhi*.
- Paratyphoid fever is clinically similar but milder disease caused by *Salmonella paratyphi*. **Enteric fever** is the general term, which includes **both typhoid and paratyphoid fever**.

Etiology

Causative agent: Enteric fevers are caused by *Salmonella typhi* and *Salmonella paratyphi*. *Salmonella* are gram-negative, motile bacilli (rods). Boiling or chlorination of water and pasteurization of milk destroy the bacilli.

Source of infection: **Humans** are the only natural reservoir and includes:

1. **Patients suffering from disease:** Infected urine, feces, or other secretions from patients.
2. **Chronic carriers of typhoid fever:** *S. typhi* or *S. paratyphi* colonizes in the **gallbladder** or biliary tree → may be associated with gallstones and the chronic carrier state.

Mode of transmission: From **person-to-person contact**.

- **Ingestion of contaminated food** (especially dairy products) and shellfish or **contaminated water**.
- **Direct spread:** Rare by **finger-to-mouth contact** with feces, urine, or other secretions is rare.

Incubation period: Usually 7–14 days.

Pathogenesis (Fig. 18.14)

Q. Write short note on pathogenesis of typhoid fever.

- The typhoid bacilli (*Salmonella*) are **ingested through contaminated food or water** → able to **survive in gastric acid of the stomach** → reach mucosa of small intestine.
- **Events during incubation period:**
 - During the initial asymptomatic period (about 2 weeks), the *Salmonella* **attach to the microvilli** and penetrate the **ileal mucosa** of the small intestine → reach lamina propria and submucosa.
 - *Salmonella* are **phagocytosed by the macrophages** (mononuclear phagocytes) present in the lymphoid tissue and **Peyer's patches** in the submucosa of small intestine.
 - *Salmonella* **multiply within the macrophages** and are **carried to the mesenteric lymph node** via lymphatics. They multiply in the lymph nodes and

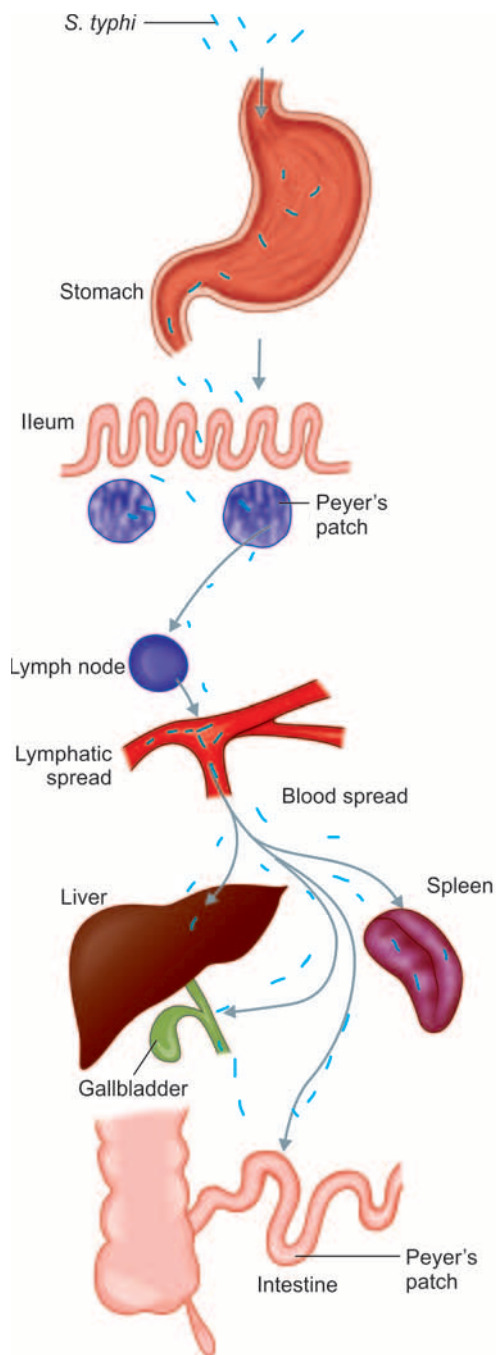


Fig. 18.14: Pathogenesis of typhoid fever

via the **thoracic duct** enter the **bloodstream** causing **transient bacteremia**.

- During bacteremia, the bacilli are **seeded in many organs**. They colonize reticuloendothelial tissues (**liver, gallbladder, spleen, bone marrow**), where bacilli multiply further causing **massive bacteremia** (occurs towards the end of incubation period) → disease clinically manifests.

- **Bile is a good culture medium for the typhoid bacillus**, and bacilli **multiply in the gallbladder** → bacilli are continuously shed through the bile into the → intestine.
- In the intestine, the bacilli are localized to the **Peyer's patches and lymphoid follicles** of the terminal ileum. They cause inflammation, plateau-like elevations of Peyer's patches and necrosis, which results in characteristic **oval typhoid ulcers**.

Typhoid fever: Most common cause of ileal perforation in tropical countries, e.g. India.

Typhoid fever: Ulceration and necrosis of ileocecal Peyer's patches.

Typhoid ulcer: Ulcers are parallel to the long axis of gut.

Typhoid fever: Perforation usually occurs during third week.

Typhoid fever: Lipopolysaccharide endotoxin is responsible for leukopenia and splenomegaly.

Typhoid fever: Relative bradycardia despite high fever.

Typhoid Mary was a cook and carrier of typhoid and infected many individuals through food.

Enteric fever caused by:

1. ***Salmonella typhi*** or
2. ***Salmonella paratyphi***.

Gallbladder colonization with *S. typhi* or *S. paratyphi* may be associated with chronic carrier state.

MORPHOLOGY**Q. Morphology of intestine in typhoid fever.**

Lesions may be 1) intestinal and 2) extraintestinal.

Intestinal Lesions**Gross (Fig. 18.15)**

- **Site:** Most commonly involved is **terminal ileum**, but may be seen in the jejunum and colon.
- **Appearance:** Peyer's patches in the terminal ileum enlarge into sharply delineated, plateau-like elevations → shedding of mucosa produces → typhoid ulcers. **Characteristics of typhoid ulcer:**
 - **Number:** Varies.
 - **Orientation:** Oval ulcers oriented along the long axis of the bowel (tuberculous ulcers of small intestine are transverse).
 - **Base of the ulcer:** It is **black** due to sloughed mucosa.
 - **Margins:** **Slightly raised** due to inflammatory edema and cellular proliferation.
 - **No fibrosis:** Hence, narrowing of the intestinal lumen seldom occur in healed typhoid lesions.

Microscopy

- **Mucosa:** Oval ulcers over the Peyer's patches.
- **Lamina propria:**
 - **Macrophages** containing bacteria, red blood cells (erythrophagocytosis) and nuclear debris
 - **Lymphocytes and plasma cells**
 - **Neutrophils** within the superficial lamina propria.

Extraintestinal Lesions

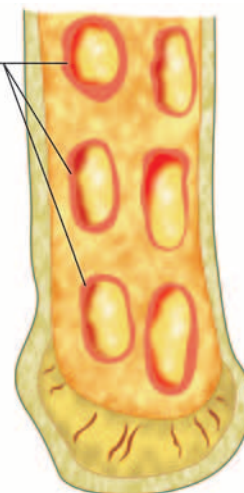
- **Typhoid nodules:** Systemic dissemination of the bacilli leads to **formation of focal granulomas** termed **typhoid nodules**. These nodules are composed of **aggregates of macrophages** (typhoid cells) containing ingested bacilli, red blood cells and lymphocytes. They are **common in the lymph node, liver, bone marrow and spleen**.
- **Mesenteric lymph nodes:** They are enlarged due to accumulation of macrophage, which contains typhoid bacilli.
- **Liver:** It shows small, scattered foci of hepatocyte necrosis replaced **typhoid nodules**.
- **Spleen:** It is enlarged and soft.
- **Abdominal muscles:** Zenker's degeneration.
- **Gallbladder:** Typhoid cholecystitis.

Clinical Features**Enteric fever:**

- Abdominal pain (hallmark symptom)
- Step-ladder fever
- Rose spots.
- Onset is gradual and patients present with anorexia, abdominal pain, bloating, nausea, vomiting and diarrhea.
- **Fever: Continuous rise in temperature** (step-ladder fever).
- **Rose spots:** These are small erythematous maculopapular lesions on the skin that fade on pressure appear on the chest and abdomen, which occur during second or third week.
- **Spleen:** It is soft and palpable may be accompanied by hepatomegaly.



Longitudinal typhoid ulcers involving Peyer's patches



Sickle cell disease: Patients are susceptible to **salmonella osteomyelitis**.

Typhoid fever: Erythrophagocytosis is a characteristic feature.

Typhoid ulcers: Oval oriented along the long axis of the bowel whereas tuberculous ulcers of small intestine are transverse.

Typhoid ulcer: Most commonly involves terminal ileum.

Figs 18.15A and B: Gross appearance of typhoid ulcers in the ileum of small intestine: (A) Specimen; (B) diagrammatic

Complications

- **Intestinal complications:** It can be fatal.
 - Perforations of ulcer
 - Hemorrhage from the ulcer.
- **Extraintestinal complications:** Encephalopathy, meningitis, seizures, endocarditis, myocarditis, pneumonia and cholecystitis. Sick cell disease → susceptible to salmonella osteomyelitis.
- **Carrier state:** Persistence of bacilli in the gallbladder or urinary tract may result in passage of bacilli in the feces or urine → causes a 'carrier state' → source of infection to others.

Complications of typhoid ulcer:

1. Perforation
2. Hemorrhage.

Typhoid fever: Perforation and hemorrhage occurs during third week.

Laboratory Diagnosis

Isolation of Bacilli

- **Blood culture:** It is positive in **first week** of fever in 90% of patients and remains positive in second week till the fever subsides. Blood culture rapidly becomes negative on treatment with antibiotics.
- **Stool cultures:** It is almost as valuable as blood culture and become positive in the **second and third weeks**.
- **Urine culture:** It reveal the organism in approximately 25% of patients by **third week**.

Other Tests

- **Widal reaction:** Classic Widal test measures antibodies against O and H antigens of *S. typhi* but lacks sensitivity and specificity. Widal test (immunological reactions) become positive from end of the first week till fourth week. There are many false-positive and occasional false-negative Widal reactions.
- **Other serologic tests:** They are available for the rapid diagnosis of typhoid fever with a higher sensitivity.
- **Total leukocyte count:** It shows **leukopenia with relative lymphocytosis**. Eosinophils are usually absent.

Absolute lymphocytosis is NOT seen in enteric fever.

Enteric fever:

1. Blood culture
2. Stool culture
3. Urine culture
4. Widal test
5. Total WBC count.

INTESTINAL TUBERCULOSIS

Q. Describe the morphology of tuberculosis of small intestine.

There are three main forms of intestinal tuberculosis, namely:

1. Primary
2. Secondary
3. Hyperplastic cecal tuberculosis.

Primary Intestinal Tuberculosis

Uncommon in the developed countries, but common in developing countries including India.

Mode of Infection

- In the pre-pasteurization era, ingestion of cow's unpasteurized milk infected with *Mycobacterium bovis*.
- Nowadays due to eradication of tuberculosis in cattle and pasteurization of milk, all intestinal tuberculosis are caused by *Mycobacterium hominis*.

MORPHOLOGY

Predominant changes are in the **regional mesenteric lymph nodes without any significant intestinal lesion**.

Gross

- **Lymph nodes are enlarged, matted and caseous (tabes mesenterica)** → may heal by fibrosis and calcification.

Microscopy

- Primary complex shows a focus in the intestinal mucosa and mesenteric lymph nodes show typical **tuberculous granuloma** with caseation necrosis (refer Fig. 16.19).

Secondary Intestinal Tuberculosis

Mode of Infection

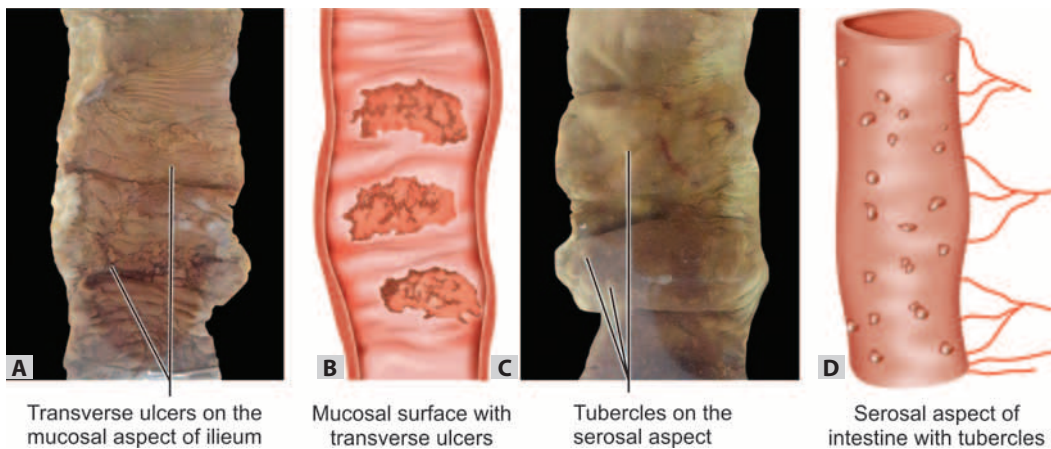
- Swallowing of sputum in patients with active pulmonary tuberculosis. The lesions are most **common in the terminal ileum**.

MORPHOLOGY

Intestinal tuberculosis: Most common site is terminal ileum and ileocecal junction.

Gross (Fig. 18.16)

- **Lesions of intestine are prominent than that of regional lymph nodes.**
- **Large ulcers** that is **transverse to the long axis of the bowel**. The surface of ulcers may be coated with caseous material. **Serosal surface may show visible tubercles** (Figs 18.16C and D).
- **Healed lesions** may show transverse **fibrous strictures** and intestinal obstruction.



Figs 18.16A to D: Ileum with tuberculous ulcer on the mucosal aspect: (A) specimen; (B) diagrammatic; Serosal aspect shows tubercles: (C) specimen; (D) diagrammatic

Ulcers of small intestine:

1. Tuberculosis
2. Typhoid
3. Crohn disease
4. Peptic.

Intestinal tuberculosis: Involved segment become thickened and serosal aspects shows tubercles.

Microscopy

- Mucosa and submucosa show ulceration and typical granulomas of tuberculosis (refer Fig. 1.20). The muscularis propria may show variable degree of fibrosis.

Hyperplastic Cecal Tuberculosis

Variant of secondary tuberculosis.

MORPHOLOGY

Gross

- Lesions are seen mainly in cecum and/or ascending colon → thickening of wall and mucosal ulceration. Clinically, the lesion present as a palpable and may be mistaken for carcinoma.

Microscopy

- It shows caseating tubercles and should be distinguished from Crohn disease in which granulomas are non-caseating.

Differences between typhoid and tuberculous ulcers are listed in Table 18.6.

SHIGELLOSIS-BACILLARY DYSENTERY

Bacillary dysentery is a necrotizing infection of the distal small bowel and colon caused by *Shigella*.

Etiology

- Shigella* causes bacillary dysentery and is one of the most common causes of bloody diarrhea.

TABLE 18.6: Differences between typhoid and tuberculous ulcers

Q. List the differences between tuberculous and typhoid ulcer of intestine.

Characteristics	Typhoid ulcer	Tuberculous ulcer
Site	Small intestine, involves Peyer's patches	Anywhere in the intestine but common in the ileocecal region
Orientation	Longitudinal to the long axis, because it involves Peyer's patch	Transverse to the long axis, because it spreads through lymphatics, which are arranged circumferentially
Microscopy	Macrophages and lymphocytes and plasma cells	Granulomas composed of central area of caseous necrosis, surrounded by epithelioid cells, Langhans giant cells and lymphocytes
Differential count	Neutropenia	Lymphocytosis
Intestinal complications		
Perforation	Common as it is an acute process associated with thinning of bowel wall	Not seen, because chronic inflammation results in healing by fibrosis
Hemorrhage	Common	Does not occur
Obstruction	Not seen	Stricture formation leads to obstruction
Extraintestinal	May develop carrier state	May develop tuberculous peritonitis

Tuberculous ulcer of intestine: Stricture formation can lead to obstruction.

- *Shigella* is an unencapsulated, nonmotile, facultative anaerobic gram-negative bacilli. They belong to the Enterobacteriaceae and are closely related to enteroinvasive *E. coli*.
- *Shigella* species that cause colitis are classified into four major subgroups, namely: *S. dysenteriae* (most virulent), *S. flexneri*, *S. boydii*, and *S. sonnei*.

Source of infection: Humans are the only natural reservoir.

Mode of transmission: By **ingestion** through fecal–oral route or via fecally contaminated water and food. It can be acquired by oral contact with any contaminated surface (e.g. clothing, towels, or skin surfaces).

Incubation period: It ranges from 1 to 3 days.

Pathogenesis

Shigella: Most virulent enteropathogens produce toxin that has cytotoxic, neurotoxic and enterotoxic effects.

- *Shigella* is the **most virulent** enteropathogens and ingestion of few (10–100 organisms) produce disease.
- After ingestion, *Shigella* reaches the stomach. They are **resistant to the action of acid in the stomach**. Small intestinal infections do not occur unless the patient has disturbance in motility of intestine.
- **In the colon**, the bacteria penetrate the intestinal mucous epithelium and are **taken up by M or microfold epithelial cells** → proliferate inside the cytoplasm of these epithelial cells → penetrate into the lamina propria → phagocytosed by macrophages → *Shigella* induces apoptosis of macrophages → causes inflammatory reaction → loosen the intercellular barriers and damages surface epithelium → leading to superficial ulcers → allows entry of *Shigella* in the intestinal lumen to the colonocyte basolateral membrane.
- *Shigella* **produces a toxin** that has cytotoxic, neurotoxic, and enterotoxic effects. When inflammation is severe, ileus, toxic megacolon, gross hemorrhage and perforation may develop.

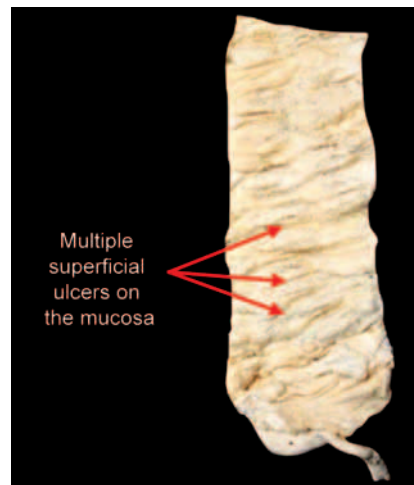
Bacillary dysentery:

- Ulcers are continuous, diffuse and perpendicular to the long axis of the colon
- Most prominent in the left colon.

MORPHOLOGY

Gross (Fig. 18.17)

- **Site of lesions:** Most prominent in the **left colon** mainly in the **rectosigmoid area**. Mostly the lesions are **continuous and diffuse**.



Bacillary dysentery:
Predominantly involves mucosa of left colon and rectosigmoid area.

Ulcers of large intestine:

1. Bacillary
2. Amebic
3. Ulcerative colitis
4. Crohn disease.

Fig. 18.17: Bacillary dysentery. Specimen of cecum and ascending colon (with appendix). Mucosa shows multiple superficial ulceration with intervening normal mucosa

- **Characteristics of ulcer:** Mucosa shows **edema, ulceration** and appears **friable granular and hemorrhagic**. In severe infections, a gray mucopurulent exudate covers the mucosa. **Ulcers** appear first on the edges of mucosal folds, **perpendicular to the long axis of the colon**.

Microscopy

Changes are predominant in the **mucosa**.

- **Early-stage:** It shows erosions and **small aphthous ulcers** (microulcers), with **infiltration by neutrophils** below the microulcers.
- **Advanced stage:**
 - **Necrosis of epithelial cells** and the damaged **mucosa is covered by purulent exudate** (composed of detached epithelial cells, neutrophils and red blood cells).
 - **Ulcers:** They are **superficial**, or may extend down to the **muscularis mucosae**. May show superficial crypt abscess, edema and mild plasma cell infiltrates.

Bacillary dysentery: Superficial ulcers with infiltration by neutrophils.

Clinical Features

- Presents as diarrhea, fever, and abdominal pain.
- The initial watery diarrhea progresses to a dysenteric phase.
- Stool culture is required for confirmation of *Shigella* infection.

Bacillary dysentery: Most common cause of bloody diarrhea caused by *Shigella*.

Complications

They are **uncommon**.

- **Reiter syndrome:** A triad of **sterile arthritis, urethritis and conjunctivitis**.
- **Hemolytic-uremic syndrome (HUS):** It is **due to toxin** secreted by *Shigella* organisms.

Complications of bacillary dysentery:

1. Reiter syndrome
2. Hemolytic- uremic syndrome.

AMEBIASIS

Q. Write short note on amebic ulcer of intestine.

Amebiasis is an **infection caused by protozoan *Entamoeba histolytica*** (named so because of its lytic actions on involved tissue).

Amebiasis: Caused by *Entamoeba histolytica*.

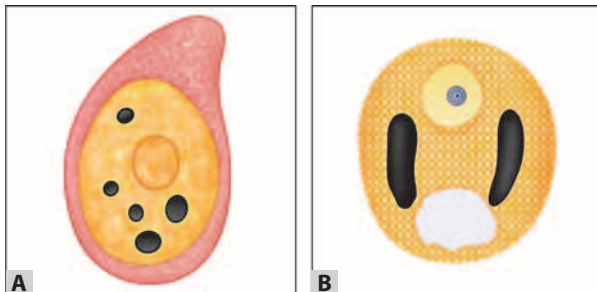
Etiology

E. histolytica: Three stages—

1. Trophozoite
2. Precyst
3. Cyst.

E. histolytica has three distinct stages:

1. **Trophozoite stage** (Fig. 18.18A): Amebic trophozoites are spherical or oval. They are seen in the stools of patients with acute symptoms. The cytoplasm of the trophozoites stains positively by PAS.
2. **Precyst stage:** In the colon, the trophozoite develops into a cyst through an intermediate form termed the precyst.
3. **Cyst stage** (Fig. 18.18B): Amebic cysts are the **infecting stage** and are found only in stools. They are spherical and have thick chitin walls and usually four nuclei.



Figs 18.18A and B: (A) Trophozoite and (B) cyst of *E. histolytica*

Source of infection: **Humans** are the only known reservoir for *E. histolytica*. It is reproduced in the colon of infected individual and passes in the feces.

Mode of infection: It is **acquired by fecal–oral route** through ingestion of materials contaminated with human feces containing *E. histolytica*.

Incubation period: About 8–10 days.

Pathogenesis

The amebic cysts are passed in the stool and the **cysts can contaminate water, food, or fingers**.

- On ingestion, *E. histolytica* cysts traverse the stomach. They are **resistant to the action of gastric acid** and they pass to the colon.
- Amebic cysts then **colonize the epithelial surface of the colon and release trophozoites**. Each cyst containing four nuclei divides to form four small, immature trophozoites → which then matures to full size. In the colon, they feed on bacteria and human cells. They may colonize any part of the large intestine, but **most frequently in the cecum and ascending colon causing amebic colitis**. These patients pass both cysts and trophozoites in the stool.
- **Trophozoite attaches the colonic epithelial cell and invades crypts, and burrow laterally into the lamina propria**. The trophozoite kills the intestinal cell by producing a lytic protein that damages the cell membrane and creates a superficial ulcer. Recruitment of neutrophils causes tissue damage and creates a **flask-shaped ulcer** with a narrow neck and broad base.
- **Trophozoite may penetrate blood vessels and reach the liver** to produce **abscesses** in about 40% of patients with amebic dysentery.

Amebiasis: Most frequently involve cecum and ascending colon.

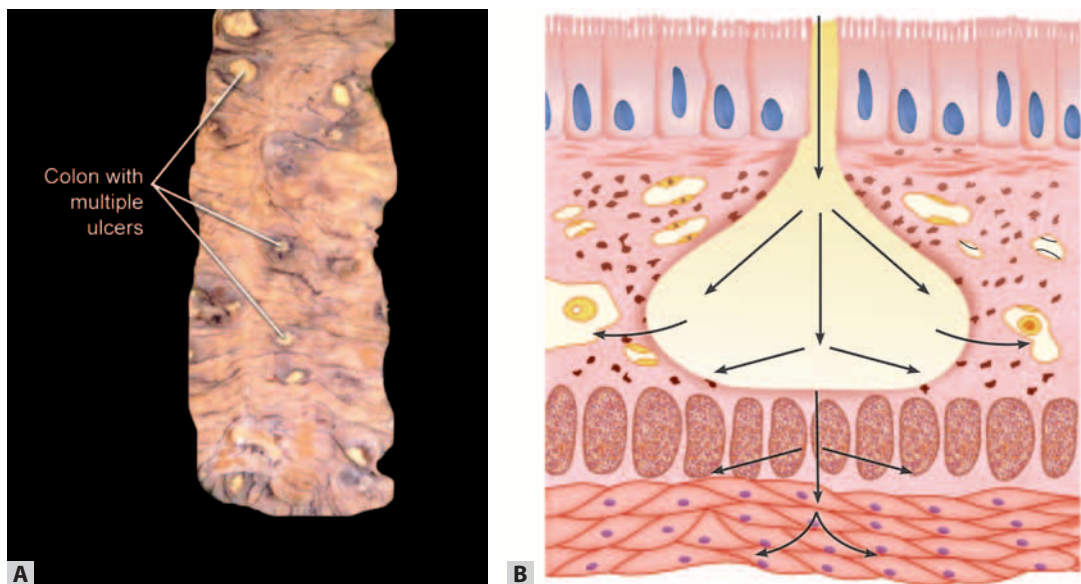
MORPHOLOGY

E. histolytica can produce intestinal or extraintestinal disease.

- **Intestinal disease:** It mainly involves the **colon** and ranges from **asymptomatic colonization to severe invasive infections** causing bloody dysentery.
- **Extraintestinal disease:** It can produce **amebic liver abscesses**.

Colon

- Amebic lesions start as small **foci of necrosis**, which progress to **ulcers**. The floor ulcer is **gray and necrotic**. The **chronic amebic ulcers** described as **flask-shaped ulcer** with a narrow/ bottle neck and broad base resembling a **flask** (Fig. 18.19A and B).
- Trophozoites can be detected on the surface of the ulcer and in the exudate. The ulcers show infiltration by acute and chronic inflammatory cells (Fig. 18.20).



Figs 18.19A and B: (A) Gross appearance of multiple amebic ulcers in the colon; (B) Microscopically appear as flask shaped amebic ulcer (diagrammatic)



Fig. 18.20: Microscopic appearance of amebic ulcer (PAS stain) shows PAS stained trophozoites of ameba in the exudate (inset)

Ameboma: It is a rare complication of amebiasis. The intestinal wall shows thickening due to inflammation (napkin-ring constriction) and may resemble colon cancer. Microscopically, it consists of granulation tissue, inflammatory cells, fibrosis and clusters of trophozoites.

Amebic ulcers: Flask-shaped ulcers in large intestine.

E. histolytica: Trophozoite invades crypts and burrow laterally in the lamina propria to produce flask-shaped ulcer.

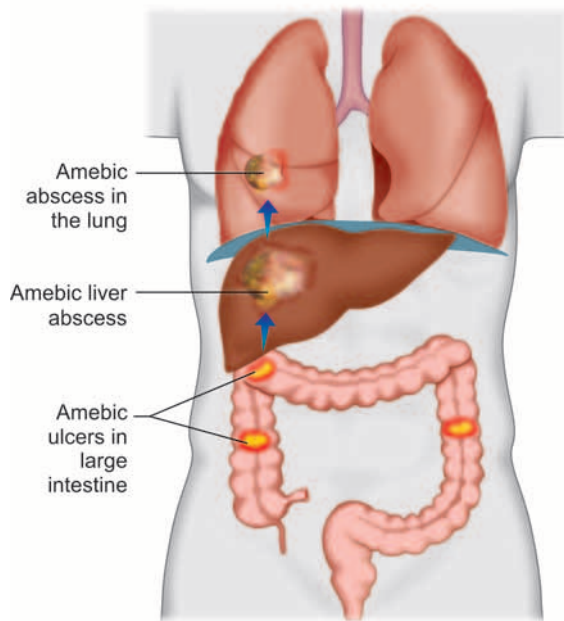
Clinical Features

Intestinal amebiasis: It may be asymptomatic or to produce dysentery of varying severity. The incubation period for acute amebic colitis is about 8 to 10 days. Amebic dysentery may present with **abdominal pain, bloody diarrhea, or weight loss**. Liquid stools (up to 25 a day) contain blood and mucus.

Amebic Liver Abscess (Fig. 18.21)

It is a **major complication of intestinal amebiasis**.

- *E. histolytica* trophozoites from the colon may reach liver through the portal circulation.
- Trophozoites kill hepatocytes → produce abscess.
- Abscess cavity is filled with a dark brown, odorless, semisolid necrotic material, which resembles **anchovy paste (sauce) in color and consistency**. The size of amebic liver abscess may vary and can exceed 10 cm in diameter.
- Spread of amebic liver abscess:
 - **Local spread:** It may expand and rupture through the capsule of the liver → may directly spread into the peritoneum, diaphragm, pleural cavity, lungs, or pericardium.
 - **Hematogenous spread:** Rare → to the brain and kidneys → necrotic lesions.



Amebic liver abscess: Anchovy sauce pus.

Fig. 18.21: Complications of amebiasis (diagrammatic)

Clinical features of amebic liver abscess: It may present with severe right upper quadrant pain, low-grade fever and weight loss. The diagnosis is usually made by radiologic or ultrasound demonstration of the abscess, in conjunction with serologic testing for antibodies to *E. histolytica*.

CARCINOID TUMOR

Q. Write short note on carcinoid tumor.

Origin: Carcinoid tumors arise from **neuroendocrine organs** (e.g. endocrine pancreas) and **neuroendocrine-differentiated epithelial cells** of GI tract (e.g. G-cells). They were originally termed carcinoid, or “carcinoma-like,” because these tumors were thought to be more indolent clinical course than GI carcinomas.

Carcinoid (carcinoma-like): Term was used because they were thought to be more indolent than GIT carcinomas.

Now these tumors are properly termed as **well-differentiated neuroendocrine tumors**.

Sites of Carcinoid Tumors

Appendix: Most common site for carcinoid tumor.

- **GI tract:** Major site
 - **Small intestine** (more than 40%) mostly in appendix.

- **Stomach:** Gastric carcinoids may be associated with endocrine cell hyperplasia, chronic atrophic gastritis and Zollinger-Ellison syndrome.

• Tracheobronchial tree and lungs.

Carcinoid tumors: Those locate in—

- Small intestine tend to be malignant
- Appendix almost always benign course.

MORPHOLOGY (FIG. 18.22)

Carcinoid tumors: Yellow in color.

Gross

- Carcinoids are **intramural or submucosal masses** → small polypoid lesions
- Mucosa covering the tumor may be intact or ulcerated
- **Yellow or tan color**
- **Very firm in consistency** due to intense desmoplastic reaction
- May invade the wall deeply to involve the mesentery.

Microscopy

- Composed of **uniform cells** → forming **islands, trabeculae, strands, glands, or sheets**.
- Cells have **scant, pink granular cytoplasm** and a **round to oval stippled nucleus**.
- Most tumors show **minimal pleomorphism** but rarely may show **anaplasia, mitotic activity and necrosis**.

Immunohistochemical stains:

- Tumor cells are positive for endocrine granule markers, such as **synaptophysin** and **chromogranin A**.

Carcinoid tumor: Positive for synaptophysin and chromogranin A.

Carcinoid tumor: Malignant potential depends on—

1. Location (most important prognostic factor)
2. Size
3. Depth of invasion
4. Growth pattern.

Clinical Features

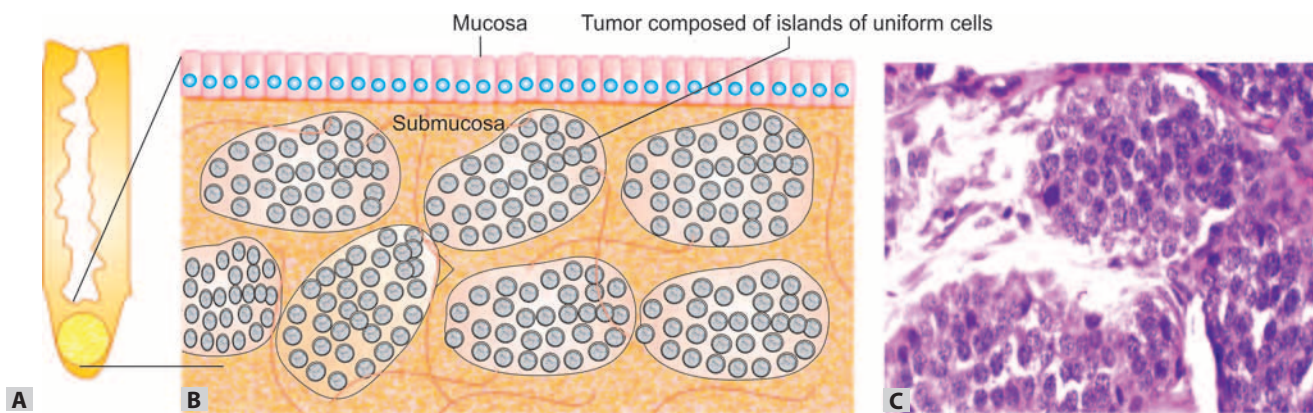
Age: Peak incidence in the **sixth decade**, but can occur at any age.

Symptoms depend on the hormones produced. For example, if the tumors produce **gastrin** → may cause **Zollinger-Ellison syndrome**. Ileal tumors → may produce **carcinoid syndrome**.

Carcinoid Syndrome (Fig. 18.23)

Q. Write short note on carcinoid syndrome.

- Develops in less than **10% of patients**.



Figs 18.22A to C: Carcinoid tumor of appendix: (A) Longitudinal section of appendix with carcinoid tumor at the tip; (B) Microscopically shows uniform cells with round to oval stippled nuclei; (C) Photomicrograph of carcinoid tumor

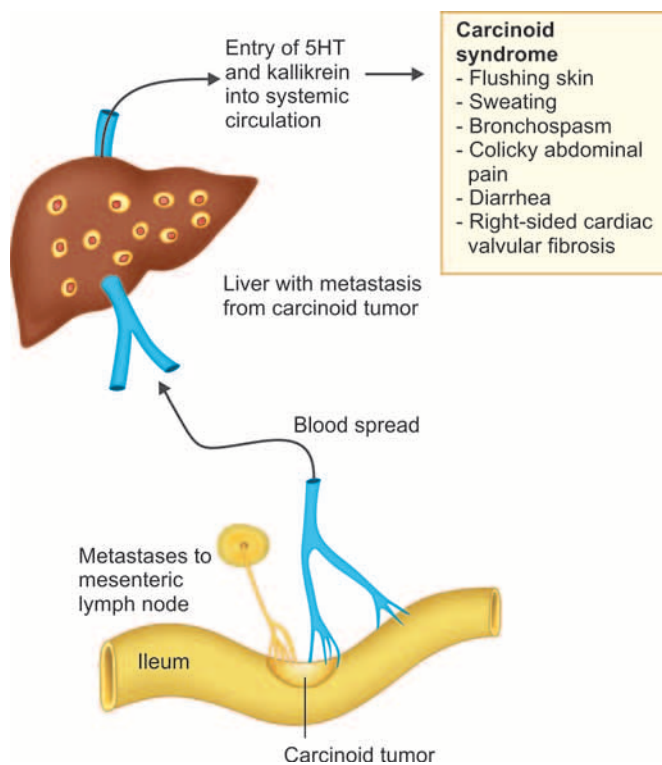


Fig. 18.23: Mechanism of development of carcinoid syndrome and its clinical features

Distribution of carcinoid tumor: Bronchus > Ileum > Rectum > Appendix > Colon > Stomach.

Foregut carcinoid:

Most common location is stomach

Mostly argyrophilic (silver staining only with the addition of reducing agent)

Produce low levels of serotonin.

Midgut carcinoid: Argentaffinic.

Small bowel carcinoids: Multiple in 25% of cases.

Hindgut carcinoid: Mixed (60–70% argyrophilic and 8–16% argentaffinic).

Appendiceal carcinoids: Typically solitary.

Carcinoid syndrome: Clinical symptoms are due to vasoactive substances secreted by tumor cells.

- Symptoms are due to vasoactive substances secreted by the tumor.
- Carcinoid tumors confined to the intestine, secrete vasoactive substances, which are metabolized to inactive forms by the liver.
- Carcinoid syndrome develops when tumors secrete hormones into a non-portal venous circulation. Therefore, it is strongly associated with metastatic disease.
- **Clinical features:** Flushing of skin, sweating, bronchospasm, colicky abdominal pain, diarrhea, and right-sided cardiac valvular fibrosis.
 - Location is the most important prognostic factor for GI carcinoid tumors.
- **Foregut carcinoid tumors**
 - Sites: Stomach, duodenum and esophagus
 - Metastasis rare and are cured by resection.

- **Midgut carcinoid tumors**
 - **Sites:** Jejunum and ileum
 - May be multiple and aggressive
 - Deep local invasion, increased size and presence of necrosis and mitosis are associated with poor prognosis.
- **Hindgut carcinoids**
 - **Sites:**
 - ♦ Appendix: Usually seen at the tip and are less than 2 cm in diameter and are benign.
 - ♦ Rectal carcinoid: It tends to produce polypeptide hormones, but metastasis is uncommon.

Malignant neuroendocrine tumors may produce carcinoid syndrome.

Carcinoid tumor syndrome: Develops with metastasis to liver/lung.

Carcinoid syndrome: Flushing, diarrhea, wheezing and sweating.

INFLAMMATORY BOWEL DISEASE

IBD: Immune-mediated chronic intestinal inflammatory condition.

- Inflammatory bowel disease (IBD) is an **immune-mediated chronic intestinal inflammatory condition**. It results from **inappropriate mucosal immune activation**.
- **Major types of IBD:** 1) **Ulcerative colitis (UC)** and 2) **Crohn disease (CD)**.

IBD:
Ulcerative colitis
Crohn disease.

Epidemiology

- **Age of onset:** Both UC and CD occurs between **15 and 30 years** of age. A second peak is between the ages of **60 and 80 years**.
- **Sex:** Male to female ratio in—
 - Crohn disease is 1.1–1.8:1
 - Ulcerative colitis is 1:1.
- **Prevalence** of IBD is **higher in urban than rural areas**, and **higher prevalence in high socioeconomic classes** than lower socioeconomic classes.
- **Cigarette smoking:** Risk of UC in smokers is 40% more than that of nonsmokers. Smoking is associated with a two-fold increased risk of CD.
- **Oral contraceptives** use: Increased risk of CD.
- **Appendectomy:** It is **protective against UC** but is associated with a **higher risk of CD**.

Etiology and Pathogenesis

Q. Describe the etiopathogenesis of inflammatory bowel disease.

IBD is an **idiopathic disorder**. The exact trigger for inflammatory bowel disease is not known. Present evidences suggest that IBD represents the outcome of three main interactive factors: **Genetic, environmental and host factors**.

Etiology of IBD:

- Genetic factors
- Environmental factors
- Host factors.

Genetic Factors

Genetic **predisposition/susceptibility** contributes to IBD.

Familial

IBD is **more common among relatives of patients with IBD** than in the general population. However, in ~ 85–90% of cases, IBD develops as sporadic disease.

- **Crohn disease:** Genetic factors **play a prominent role**. The concordance rate for monozygotic twins is about 50%.
- **Ulcerative colitis:** Genetic factors are **less prominent than in Crohn disease**. The concordance of monozygotic twins is only 16%. Concordance for dizygotic twins for both Crohn disease and ulcerative colitis is less than 10%.

Susceptibility Genes

IBD susceptibility genes:

- *NOD2/CARD15*
- *ATG16L1*
- *IRGM*.

- **Genes associated with innate immunity and autophagy** (e.g. *NOD2/CARD15*, *ATG16L1* and *IRGM*): **They respond to infection and clear the infective agents** such as bacteria, mycobacteria and viruses. *NOD2/CARD15* is the first Crohn disease susceptibility gene was originally known as *NOD2* (nucleotide oligomerization binding domain 2) but subsequently renamed as *CARD15*. ***NOD2/CARD15* gene codes for a *NOD2/CARD15* protein, which is required for normal recognition and response against microbes in the intestinal lumen.** Actions of *NOD2/CARD15* protein are:
 - **Prevents the entry of microbes** into the wall of the intestine
 - **Regulates innate immune responses**
 - **Prevents excessive immune response** against luminal microbes.

- **Defective NOD2/CARD15:** It is produced due to **mutations** and mutant forms, are less effective at recognizing and combating luminal microbes. Defective NOD2:
 - Allows the entry of luminal microbes into the lamina propria of the intestinal wall and stimulates inflammatory reactions.
- **Two other genes** namely *ATG16L1* (autophagy-related 16-like) and *IRGM* (immunity-related GTPase M) are involved in the recognition and response to intracellular pathogens.

NOD2/CARD15 is on chromosome 16q, also known as IBD-1 locus.

Environmental Factors

Environmental factors in IBD:

- Intestinal microflora
- Smoking.

Includes both the local microenvironment (intestinal microflora) and the nutritional environment.

Intestinal Microflora/Microbiota

- **Hygiene hypothesis:** The **gut lumen** contains **abundant commensal bacteria** and its composition within individuals remains stable for several years. This intestinal microflora **can be modified by diet and disease**. IBD is associated with an alteration in the bacterial flora.
- **Improved food storage conditions and decreased food contamination:** It has **reduced frequency of enteric infections** with 'clean' environment in the intestinal lumen → the **immune system may not be exposed to microorganisms** (pathogenic or nonpathogenic) → **inadequate development of regulatory processes** to limit mucosal immune responses.
- **Inadequate immune regulation:** The '**untrained**' **immune system** when exposed to the normal commensal bacterial antigens/infections → **inadequate immune regulation** → allows pathogens (which normally cause self-limited disease) to produce an **over aggressive immune responses** and **uncontrolled chronic inflammatory response** in a genetically susceptible individual.

Smoking

CD patients are more likely to be smokers and smoking exacerbates CD. In contrast, an increased risk of UC is observed in non- or ex-smokers.

Smoking:

- Strong exogenous risk factor for development of CD
- Partly relieves symptoms in UC.

Host Factors

Host factors in IBD:

- Epithelial defects
- Defective mucosal immune response.

1. **Epithelial defects:** A variety of epithelial defects can develop in IBD.
 - **Impaired mucosal barrier function:** It is observed in IBD → **can activate innate and adaptive mucosal immunity and sensitize individuals to disease**.
 - **Abnormal intestinal defensins:** Paneth cell granules contain **antimicrobial peptides termed defensins**, which normally protect the mucosa against adherent and invading bacteria. **Abnormal defensins** are found in Crohn disease patients carrying *ATG16L1* mutations.
2. **Defective mucosal immune responses (immune dysregulation):** Immunological abnormality have been observed in both **innate** (macrophage and neutrophil) and **acquired** (T- and B-cell) **immunity**.
 - **Normal regulatory immune response of gut mucosa:** It is very powerful and **prevents immunologic/inflammatory response** against the dietary antigens and the commensal microbiota.
 - **Defective regulatory immune response:** In a genetically predisposed individual, the regulatory immune suppression is defective → leads to **abnormal immune response to nonpathogenic commensal bacteria** within the gut → **activates CD4⁺ T-cells** in the lamina propria → **secretes excess of inflammatory cytokines** relative to anti-inflammatory cytokines → **imbalance between the proinflammatory and anti-inflammatory mediators** → **uncontrolled inflammation**.

IBD: Imbalance between proinflammatory and anti-inflammatory mediators → uncontrolled inflammation.

Crohn disease: Abnormal defensins in patients with *ATG16L1* mutations.

Psychosocial Factors

It can contribute to worsening of symptoms in IBD. Major life events (e.g. illness or death in the family, divorce or separation) are associated with an increase in symptoms such as pain, bowel dysfunction and bleeding.

Hypothesis for Pathogenesis of IBD

The IBD is characterized by **abnormal mucosal immunological response** and shows lymphocytes, macrophages

and other cells of the immune system infiltrating the lamina propria. However, the antigens that trigger the immune response are not yet identified. Whatever the antigenic trigger, **activated T-cells in the lamina propria are involved in the pathogenesis of IBD.**

IBD: Three types of T_H T-cells involved—

1. T_H1 T-cell
2. T_H2 T-cell
3. T_H17 T-cell.

Hypothesis (Fig. 18.24): One hypothesis signifies the roles of intestinal microbiota, epithelial function and mucosal immunity in the pathogenesis of IBD. The various events are:

In genetically susceptible host, combination of defects in host interactions with intestinal microbiota, intestinal epithelial dysfunction and aberrant mucosal immune responses trigger IBD.

- **Trans epithelial flux of luminal bacterial components:** Bacterial components/antigens within the intestinal microbiota **pass between leaky epithelial cells** or enter the lamina propria through ulcerated mucosa.
- **Processing of bacterial antigens:** Antigens from the lumen of the bowel are **transported by M (microfold)**

and dendritic cells in the specialized (follicle associated epithelium (FAE)). **Antigen-presenting cells (APC)** in the lamina propria **process these bacterial antigens and present to $CD4^+$ T helper (T_H) cells.**

- **T-cell activation and differentiation:** $CD4^+$ T-cells are activated and undergo differentiation by the cytokines secreted by APCs. Major cytokines involved in IBD include: IL-12, IL-23, IL-1 and IL-6.
- **Role of $CD4^+$ T helper (T_H) cells:** They **promote inflammation by secreting excess of cytokines** and contribute to the pathogenesis of IBD.
 - **T_H1 cells** secrete interferon gamma ($IFN\gamma$) which is the most powerful cytokine that activates macrophages; and also TNF. In a **genetically susceptible individual**, the release of TNF and other immune-mediated signals **increases the tight junction permeability of epithelial cells**. This causes further **increase in the entry of bacterial components** into the lamina propria. These events may result in a **self-amplifying cycle** which may be sufficient to initiate IBD.
 - **T_H2 cells** secrete IL-4, IL-5 and IL-17. IL-13 **induces superficial inflammation of mucosa**.

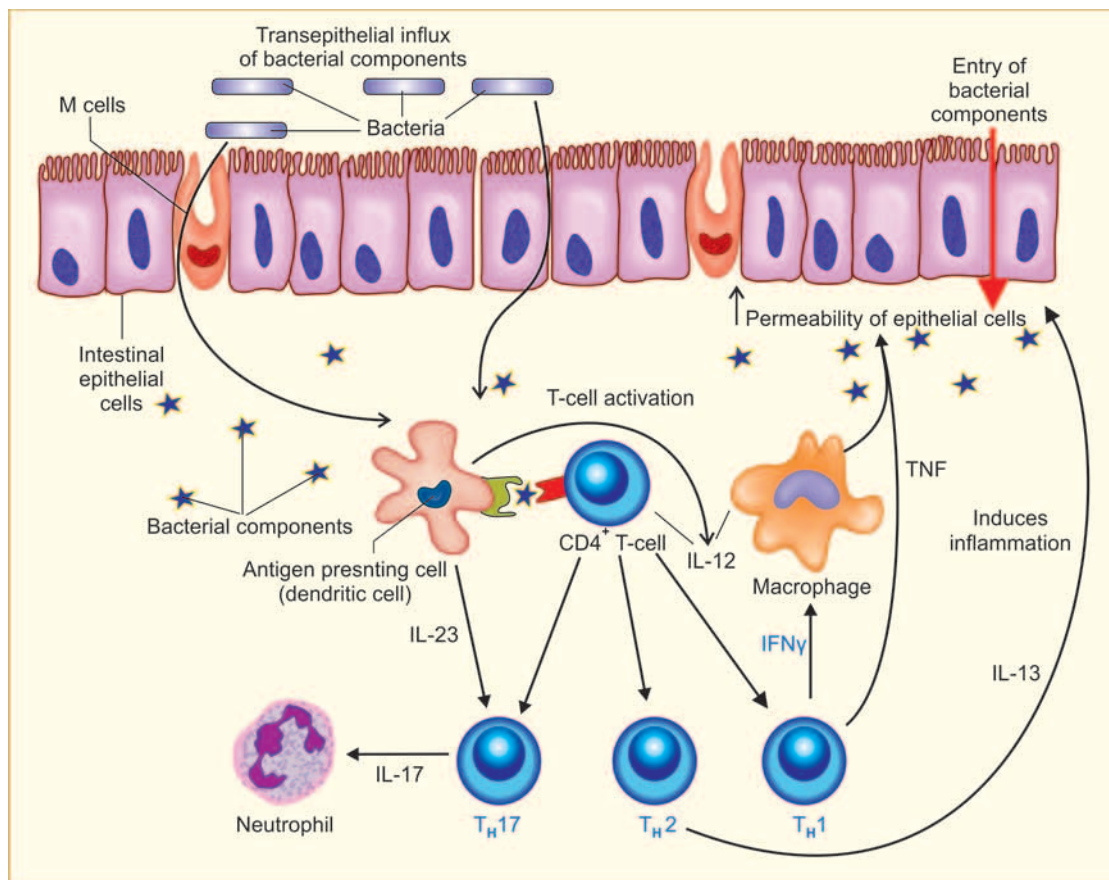


Fig. 18.24: Pathogenesis of inflammatory bowel disease (IBD)

- **T_H17 cells** secrete IL-17, IL-21 and are responsible for neutrophilic recruitment. **IL-23** secreted by APCs is essential for the **development and maintenance** of T_H17 cells.

Polymorphism of IL-23 receptor is protective in IBD.

- **Differentiation of CD4⁺ T-cells:** Cytokines secreted by APCs causes differentiation of CD4⁺ T-cells into three major types of CD4⁺ T helper effector cells, namely:
 - T_H1 type by IL-12
 - T_H2 type by IL-4
 - T_H17 type by IL-23
- **T_H1 T-cell secretion:** T-cells continue the inflammatory process with activation of non-immune cells and release of other important cytokines, e.g. interleukin (IL)-12, IL-23, IL-1, IL-6 and tumor necrosis factor (TNF).

The above mentioned pathways occur in all normal individuals exposed to an inflammatory insult. These processes are self-limiting in healthy subjects.

CROHN DISEASE

Crohn disease: Chronic progressive inflammatory bowel disease.

Crohn disease (regional enteritis) is a **chronic multifocal relapsing and remitting, progressive inflammatory bowel disease** of unknown cause that **can involve any portion of the gastrointestinal tract** (Fig. 18.25).

MORPHOLOGY

Q. Write short note on morphological features of Crohn disease.

Gross

Crohn disease: Commonly in the terminal ileum, but can involve any portion of the gastrointestinal tract.

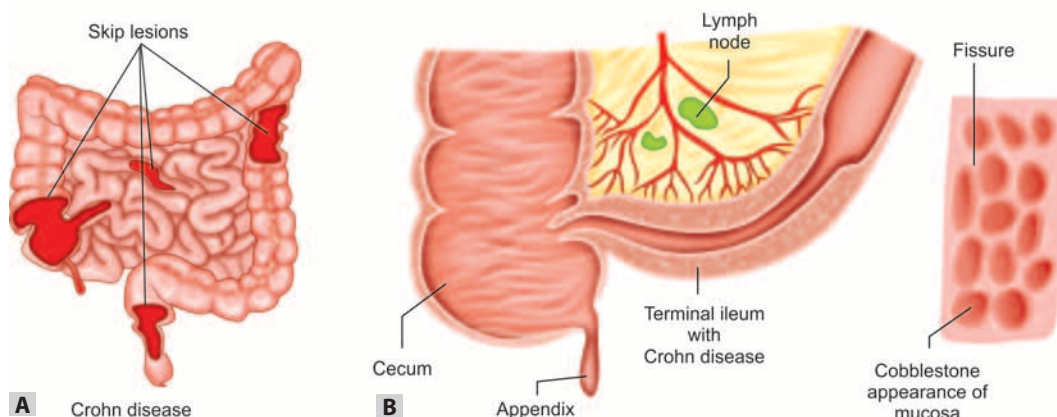
- **Sites (Fig. 18.25A):** It can involve any area of the GI tract **from mouth to anus**.
 - **Most commonly** in the **terminal ileum, ileocecal valve and cecum**.
 - Small intestine alone in ~40%; small intestine and colon in ~30% and only colon in ~30%.
- **Number of lesions:** Usually **multiple** and each lesion is **sharply demarcated** from intervening normal bowel → giving rise to **characteristic skip lesions**. It **helps in differentiating it from ulcerative colitis**.
- **Mucosal lesions:**
 - **Aphthous ulcer** is the **earliest lesion**. **Multiple lesions may coalesce** longitudinally to form → elongated, linear or **serpentine (snake-like)/train track/ rake ulcers oriented along the axis of the bowel**.
 - **Later** the ulcer become deeper and form linear clefts or **fissures** → may extend deeply to **become fistula tracts or sites of perforation**.
 - Mucosa shows edema and loss of the normal mucosal fold.
 - Ulcers may fuse longitudinally and transversely. The **islands of normal mucosa between the ulcers** show edema and produce a **cobblestone appearance** (Fig. 18.25B).
- **Intestinal wall (Fig. 18.25B):** In the **involved region**, it shows **fibrotic thickening** and is **rubbery**. The **lumen is narrowed** and strictures are common. It appears **like a hosepipe** → produces a characteristic radiological '**string sign**'.
- **External surface/serosa:** It appears red, opaque, hyperemic and covered with serosal exudate producing **serositis**. When the disease shows extensive transmural involvement, fat may encircle around the antimesenteric serosal surface producing a pattern known as **creeping fat**.
- **Adhesion:** Involved loops of bowel may adhere to each other.

Crohn disease: Radiologically characteristic string sign is due to only a trickle of contrast medium passing through the narrowed affected segment.

Microscopy (Figs 18.26A and B)

Q. Describe the gross and microscopy of Crohn disease.

Crohn disease is a **chronic inflammatory process**.



Figs 18.25A and B: (A) Distribution of lesions in Crohn disease; (B) Gross features of Crohn disease

Crohn disease: Two major characteristic features—

1. Transmural inflammation (all layers of bowel)
 2. Skip lesions (inflamed segments separated by normal intestine).
- **Mucosal ulcers and inflammation:** They appear as small, superficial called as **aphthous ulcers**. During early phases, the inflammation may be limited to the mucosa and submucosa. Both mucosa and submucosa shows edema and an increase in the number of lymphocytes, plasma cells and macrophages.
 - **Crypt abscesses:** These are characterized by the presence of numerous/**clusters of neutrophils within a crypt**. These neutrophils may infiltrate and damage crypt epithelium and are often associated with crypt destruction.
 - **Distortion of mucosal crypt architecture:** Normally, crypts are straight and parallel to each other. Repeated cycles of crypt destruction and regeneration lead to **bizarre branching shapes and unusual orientations to one another** → referred as distortion of mucosal crypt.
 - **Epithelial metaplasia:** It develops as a consequence of chronic injury.
 - **Pseudopyloric metaplasia:** It is characterized by the occurrence of **glands, which appear like** those in the **gastric antrum** may be seen in the involved segment of the intestine.
 - **Paneth cell metaplasia:** It may appear in region with metaplasia (Paneth cells are normally absent in the left colon).
 - **Noncaseating granulomas** (see Fig. 18.26): It is a **hallmark of Crohn disease**. They are **mostly seen in the submucosa** and found in ~35% of cases. These granulomas consist of focal aggregates of epithelioid cells, surrounded by a thin rim of lymphocytes. Multinucleated giant cells may also be present.
 - **Transmural inflammation:** It is characterized by **chronic inflammation involving all layers of the intestinal wall** and may show **lymphoid aggregates** in the submucosa or subserosa.

Crohn disease: Granulomas in the skin may form nodules and are called (misleading) as metastatic Crohn disease.

Mnemonic for Crohn disease: SISTER IN CCF

- S- Skip lesions
- I- Ileum is the most commonly involved
- S- String sign on radiology
- T- Transmural inflammation
- E- Extensive fibrosis
- R- Rectum usually spared
- I- Intermittent attacks with colicky abdominal pain
- N- Noncaseating granuloma
- C- Cobblestone appearance on mucosa
- C- Creeping fat on serosa
- F- Fistula may develop.

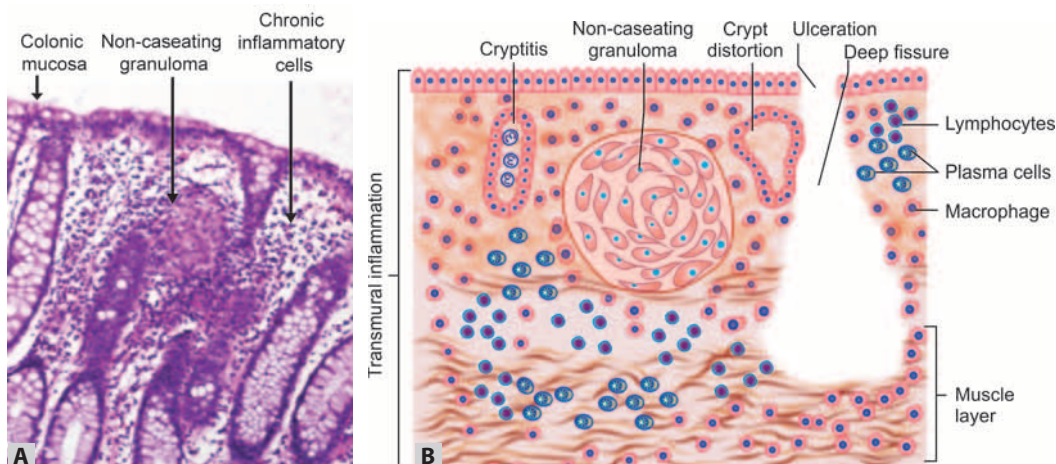
Clinical Features

Intestinal Manifestations

- Intermittent attacks of **mild diarrhea, fever and abdominal pain**.
- About 20% present with acute right lower quadrant pain, fever and bloody diarrhea that may mimic acute appendicitis or bowel perforation.
- Periods of active disease are usually interrupted by asymptomatic periods. Disease may re-activated by external triggers, such as physical or emotional stress, specific diets and cigarette smoking.

Extraintestinal Manifestations

These include **uveitis, migratory polyarthrititis, sacroiliitis, ankylosing spondylitis, erythema nodosum and clubbing of the finger**.



Figs 18.26A and B: (A) Photomicrograph of Crohn disease showing one non-caseating granuloma; (B) (Diagrammatic) Microscopic features of Crohn disease

Laboratory Findings

ESR and CRP are raised. In severe disease, there may be hypoalbuminemia, anemia and leukocytosis.

Complications

Q. Write short note on complications of Crohn disease.

1. **Iron-deficiency anemia:** It may develop in patients with colonic disease.
2. **Malabsorption:** Extensive involvement of the small intestine may result in loss of protein, hypoalbuminemia and malabsorption.
3. **Stricture formation:** It may occur in the terminal ileum.
4. **Fistula formation:** It may form between loops of intestine and surrounding structures such as urinary bladder, vagina and abdominal or perianal skin. Perforations and peritoneal abscesses are common.
5. **Acute complications:** Perforation and hemorrhage.
6. **Development of carcinoma:** It is rare and risk of carcinoma colon is increased in patients with long-standing colonic disease.
7. **Systemic amyloidosis:** It is rare.

Crohn disease: Fistulas may penetrate the bowel into other organs (e.g. bladder, uterus, vagina and skin). Lesions in the distal rectum and anus may produce perianal fistulas.

ULCERATIVE COLITIS

Q. Write short note on morphology of ulcerative colitis.

Ulcerative colitis (UC) is a **severe, chronic crypt destructive, ulcerating inflammatory** bowel disease of unknown cause. It is **limited to the colon and rectum** and inflammation involves only the **mucosa and submucosa** of the intestinal wall. It is clinically associated with exacerbations and remissions of bloody diarrhea.

Ulcerative colitis: Severe ulcerating inflammatory bowel disease.

MORPHOLOGY

Gross (Fig. 18.27)

Site: Ulcerative colitis is a **diffuse disease limited to colon and rectum**. Usually involves the rectum and extends proximally for a variable distance (continuous lesion) to involve part or the entire colon. **Skip lesions are not seen** in UC.

UC: Diffuse disease is limited to the colon and rectum. Inflammation involves only the mucosa and submucosa.

Terminology used depending on the region involved is shown in Table 18.7.

Early Active Colitis

- The **mucosal surface** may appear **slightly red** and **fine granular** that **resembles sandpaper**. It is **frequently covered with a yellowish exudate** and bleeds easily.
- **Small, superficial erosions or broad-based ulcers** may develop **later**. Ulcers are **aligned along the long axis** of the colon but do not appear like serpentine ulcers of Crohn disease.

Chronic Colitis

- **Pseudopolyps (inflammatory polyps):** They develop in long-standing disease, **isolated islands of regenerating mucosa** may **bulge into the lumen** of the bowel to produce small elevations → termed **pseudopolyps** (see Fig. 18.27).
- **Mucosa** may appear **granular or smooth**. As the disease progresses, mucosal folds are lost → **mucosal atrophy**.
- Bowel wall is **not thickened**, the **serosa is normal**, and **strictures do not develop**.
- **Inflammatory process** is **diffuse** (without skip lesion) and **limited to the mucosa and superficial submucosa**.
- In **severe cases**, inflammation may damage the muscularis propria and disturb neuromuscular function → lead to **colonic dilation** and **toxic megacolon** → can undergo perforation.
- In **severe cases**, inflammation may damage the muscularis propria and disturb neuromuscular function → lead to **colonic dilation** and **toxic megacolon** → can undergo perforation.

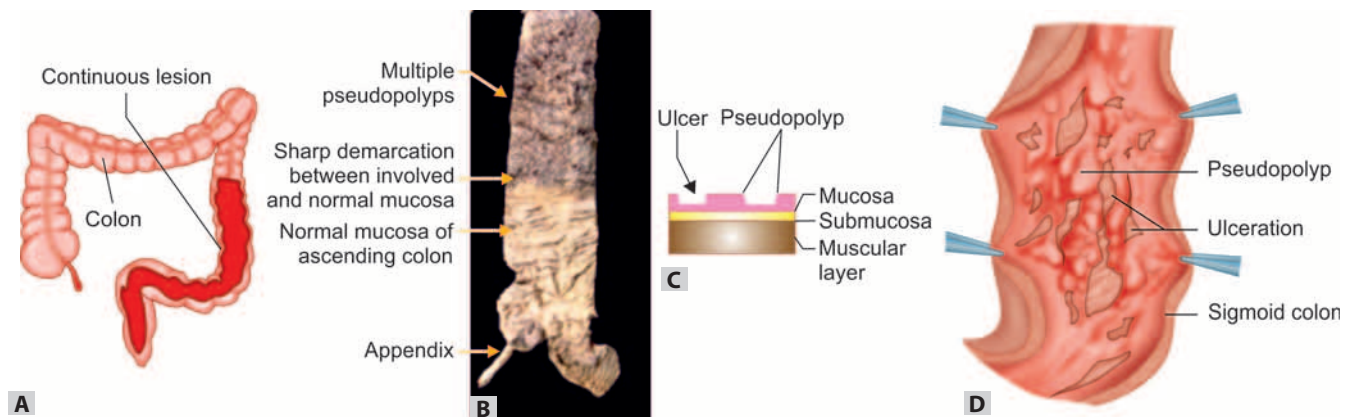
Microscopy (Box 18.4)

Early active colitis (Fig. 18.28)

- **Inflammation**
 - **Mucosal changes:** Mucosal congestion, edema and microscopic hemorrhages.
 - **Chronic inflammatory infiltrates:** It consists of **lymphocytes, plasma cells and macrophages in the lamina propria**. The density of plasma cells is more in the basilar region of the lamina propria (**basal plasmacytosis**) and extend into the muscularis mucosae.
 - **Changes in the crypt:** **Neutrophils** are the **hallmark of acute disease**. They are located in the epithelium of crypts and damage the crypt epithelium giving rise to **cryptitis** (Fig. 18.28). Clusters of neutrophils are seen within a dilated crypt and are known as **crypt abscesses** and are **usually associated with destruction of crypts**.
- **Crypt injury and architectural distortion.**

Resolution phase (Quiescent, or inactive ulcerative colitis)

- **After an attack of active colitis**, most patients enter into a **resolution phase** of decreasing activity and symptoms.
- It is characterized by **decreased activity and crypt injury**, with crypt regeneration.
- First, neutrophils and crypt injury decreases.
- Lymphocytes and plasma cells persist and are the last cells to disappear from the mucosa.



Figs 18.27A to D: Gross features of ulcerative colitis: (A) Diagrammatic; (B) Ascending colon showing numerous pseudo polyps; (C) Pseudopolyp (diagrammatic); (D) Mucosal surface of colon with pseudopolyps (diagrammatic)

Mnemonic for ulcerative colitis: COLD TIP

- C- Cryptitis, crypt abscess
- O- Originates in the rectum
- L- Limited to colonic mucosa and submucosa
- D- Distortion of crypts and depletion of mucin
- T- Toxic megacolon
- I- Increased risk of colonic cancer compared to Crohn
- P- Pseudopolyps.

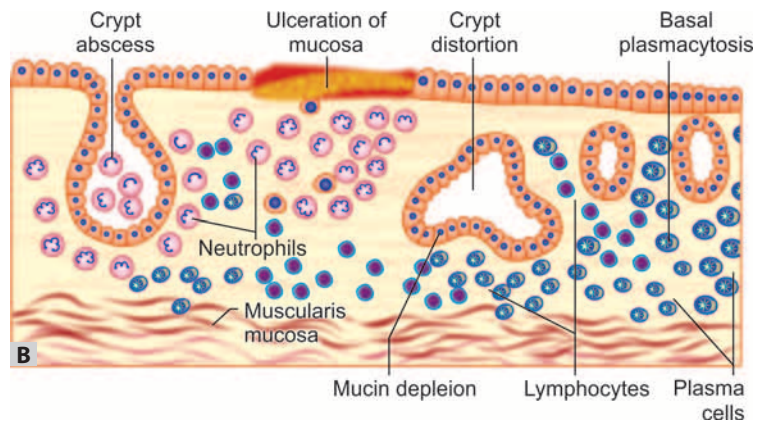
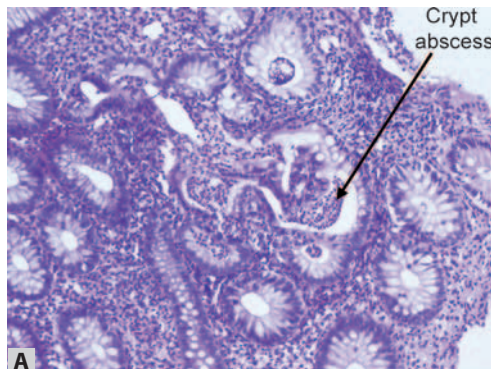
Ulcerative colitis:

Smoking appears to confer protective effect

Patients who had an appendicectomy appear to have decreased risk of UC.

Rectal involvement (proctitis) is the hallmark of UC.

UC: Inflammatory response is found only in the mucosa and submucosa.



Figs 18.28A and B: Ulcerative colitis: (A) Photomicrograph showing crypt abscess (arrow) and dense inflammatory infiltrate surrounding crypts; (B) Diagrammatic shows microscopic features of ulcerative colitis

TABLE 18.7: Terminology used for ulcerative colitis depending on the region involved

Region involved	Terminology
Entire colon	Pancolitis
Only the left-side of colon (without extension beyond transverse colon)	Left-sided disease
Limited to the rectum	Ulcerative proctitis
Disease process extends from rectum proximally towards the splenic flexure	Ulcerative proctosigmoiditis
Mild mucosal inflammation of the distal ileum	Backwash ileitis

Backwash ileitis: Ulcerative colitis involving distal ileum.

BOX 18.4: Microscopic features of ulcerative colitis

- Inflammatory infiltrates of lymphocytes, plasma cells and macrophages
- Cryptitis and crypt abscesses
- Architectural crypt distortion
- Epithelial metaplasia
- Basal plasmacytosis

Chronic ulcerative colitis

The **resolution phase** of decreased activity may last for several months. This may gradually progress into the phase of chronic ulcerative colitis (CUC).

- **Histological hallmarks of chronicity:**
 - **Distortion of crypt architecture:** It is due to repeated destruction and regeneration of crypt. The crypts may appear shortened, tortuous with bizarre branching (bifid) and decreased in number. Often a gap is seen between the crypt bases and the muscularis mucosae.
 - **Crypt architectural distortion:** The crypts may be bifid and reduced in number, often with a gap between the crypt bases and the muscularis mucosae.
 - **Chronic inflammatory infiltrate:** A chronic inflammatory infiltrate of lymphocytes, plasma cells and macrophages are seen in the mucosa and superficial submucosa.
 - **Basal plasmacytosis** and multiple basal lymphoid aggregates.
- **Mucosal atrophy:** It occurs in late stages. The mucosa surface appears smooth, flat without any normal folds.
- **Paneth cell metaplasia:** It is common, which may occur in the left colon (where Paneth cells are normally not found).
- **Loss of goblet cells and mucin depletion:** The regenerating epithelium is immature and does not contain mucin, and is called as mucin depletion. (Typical mature goblet cell contain mucin).
- **Dysplastic changes in epithelium:** It may appear and may progress to carcinoma.

Ulcerative colitis: Early acute ulcerative colitis:

1. Mucosal congestion, edema and hemorrhages
2. Cryptitis and crypt abscess
3. Chronic inflammatory cells (lymphocytes, plasma cells and macrophages) and basal plasmacytosis.

Ulcerative colitis: Granulomas and skip-lesions are not seen.

Ulcerative colitis:

Four Cs:

1. Congestion
2. Crypt abscess
3. Crypt distortion
4. Chronic inflammation.

Four Ms:

1. Mucosal atrophy
2. Mucin depletion
3. Metaplasia
4. Mucosal dysplasia.

Clinical Features

Ulcerative colitis: Exacerbations and remissions.

- Occurs mainly in young adults.
- **Triggering event:** Infectious enteritis and psychological stress.
- UC is a relapsing disorder with **exacerbations and remissions**.

Intestinal Manifestations

- Presents with **attacks of bloody diarrhea** with mucoid material, **lower abdominal pain** and cramps.

Extraintestinal Manifestations

IBD: Both CD and UC can have extraintestinal manifestations.

- **Migratory polyarthritis, sacroiliitis, ankylosing spondylitis.**
- Inflammation of eye (mostly **uveitis**).
- Skin lesions: Erythema nodosum and pyoderma gangrenosum.
- Liver disease: Primary sclerosing cholangitis and pericholangitis.
- Thromboembolic phenomena: Deep vein thrombosis of the lower extremities.

Complications**Q. Write short note on complications of ulcerative colitis.**

1. **Toxic megacolon:** In fulminant cases, the inflammation and inflammatory mediators can damage the muscularis propria and disturb neuromuscular function. This may lead to colonic dilation and toxic megacolon → may lead to perforation.
2. **Colorectal cancer:** It may develop in long-standing UC.
3. **Hemorrhage** from intestinal lesions → blood loss.
4. **Electrolyte disturbances** due to diarrhea.

Complications of ulcerative colitis:

1. Toxic megacolon
2. Development of colorectal carcinoma
3. Intestinal hemorrhage
4. Electrolyte imbalances.

Prognosis: It depends on the severity of active disease and disease duration.

Differences between ulcerative colitis and Crohn disease are listed in Table 18.8.

TABLE 18.8: Differences between Crohn disease and ulcerative colitis

Characteristics	Crohn disease	Ulcerative colitis
1. Macroscopic		
Bowel region involved	Ileum ± colon	Colon and rectum
Distribution	Skip lesions	Diffuse
Luminal narrowing/stricture	Positive	Rare
Involved bowel wall	Thick	Thin
2. Microscopic		
Inflammation	Transmural	Limited to mucosa and submucosa
Pseudopolyps	Few	Many
Ulcers	Deep, fissures	Superficial, broad-based
Lymphoid reaction	Marked	Moderate
Fibrosis	Marked	Minimal to none
Serosal inflammation	Marked	Absent
Granulomas	Seen in ~35%	Absent
Fistulae/sinuses	Seen	Absent
Crypt abscess	Uncommon	Usual
3. Clinical		
Fissure/ fistula	Observed with colonic involvement	Not seen
Fat/vitamin malabsorption	Seen	Absent
Development of malignancy	In colonic involvement	Yes
Recurrence after surgery	Common	Not observed
Toxic megacolon	No	Yes

Note: All features may not be seen in a single case.

INTUSSUSCEPTION

Q. Write short note on intussusception.

Intussusception is an **invagination of one segment of intestine into another immediately adjacent distal segment**.

Causes

Intussusception: Telescoping/invagination of one segment of intestine into another immediately adjacent distal segment.

- **Infants and children:** Usually there is **no underlying anatomic defect**.
- **Older children and adults:** A **mass or tumor in the wall of the bowel** disturbs normal peristaltic contractions forcing the lesion and a segment of proximal bowel into a distal segment of intestine. The lesion act as the point of traction and causes intussusception. These lesions **include polyps, ingested foreign bodies**, Meckel diverticulum, etc.

MORPHOLOGY

Intussusception: Three parts—

1. Intussusceptum
2. Returning or middle
3. Intussusciens.

Sites: In children, most common site is ileocolic and in adults it is colocolic.

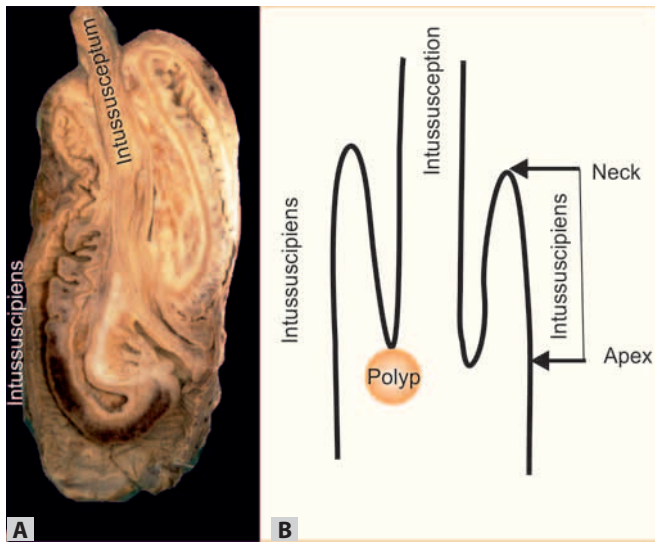
Gross (Fig. 18.29): An intussusception consists of **three parts**:

1. Entering/inner tube (intussusceptum)
2. Returning or middle tube
3. Sheath or outer tube (intussusciens).

Effect: Untreated intussusception may lead to intestinal obstruction, compression of vessels and infarction of the bowel.

POLYPS OF COLON

Definition: A gastrointestinal polyp is a **mass that protrudes into the lumen of the gut**.



Figs 18.29A and B: Intussusception: (A) Ileoileal intussusception; (B) Mechanism and nomenclature of intussusception

Intussusception: Highest incidence between 4 and 10 months of age.

Intussusception: Ileocolic is the most common type.

Sigmoid colon: Most common site for polyps and cancer.

Polyps are most common in the colon but may occur in the esophagus, stomach, or small intestine. They are of clinical importance because of their tendency to undergo malignant transformation.

Classification of Polyps (Box 18.5)

Q. Classify colorectal polyps.

BOX 18.5: Classification of gastrointestinal polyps

According to gross appearance

- Sessile: Polyps do not have a stalk
- Pedunculated: Polyps have a well-defined stalk

According to histopathological appearance

- Non-neoplastic
 - Inflammatory
 - Hamartomatous
 - Hyperplastic
- Neoplastic
 - Benign: Adenoma (Tubular, tubulovillous and villous)
 - Malignant

Non-neoplastic Polyps

Inflammatory polyps develop in: Ulcerative colitis.

Inflammatory Polyps

- These are raised nodules of inflamed, regenerating epithelium. These lesions are not precancerous and are commonly found in **ulcerative colitis** (see Fig. 18.27).
- **Microscopy:** Composed of a distorted and inflamed mucosal glands and granulation tissue.

Hamartomatous Polyps

Hamartomatous polyps: Occur sporadically or as a part of genetic diseases.

- **Rare tumor-like growths.**
- They consist of **mature tissues that are normally present at the site** in which they develop.
- They may be associated **intestinal and extraintestinal manifestations**.

Hamartoma: Jumbled mass of tissue indigenous to the part.

Hamartomas are **NOT** preneoplastic precursor lesions.

Juvenile Polyps

These are **focal hamartomatous malformations of the mucosal epithelium and lamina propria**.

- Majority occur in **children** less than 5 years of age.
- Located in the **rectum** and commonly present with rectal bleeding.

Juvenile Polyposis Coli Syndrome (JPS)

- It is characterized by multiple juvenile or inflammatory polyps distributed throughout the colon or GI tract.
- It is associated with an **increased risk of colonic adenocarcinoma**.

MORPHOLOGY

Gross

- Most are **less than 3 cm** in diameter.
- **Pedunculated, spherical, smooth-surfaced** and red in color
- Cut section shows **cystic spaces**.

Microscopy (Fig. 18.30)

- **Cystically dilated glands** filled with mucin and inflammatory debris.
- **Lamina propria** is expanded due to **mixed inflammatory infiltrates**.

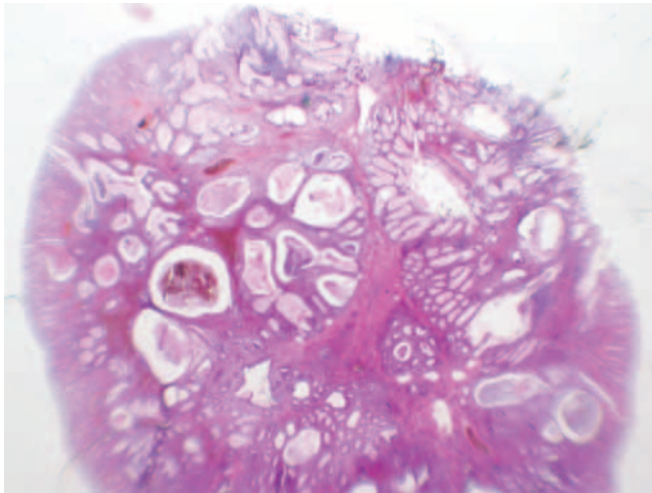


Fig. 18.30: Whole-mount view of a juvenile polyp showing cystically dilated glands

Peutz-Jeghers (PJ) Syndrome (Fig. 18.31)

Q. Write short note on Peutz-Jeghers syndrome.

- Rare **autosomal dominant** syndrome
- **Median age** of presentation is **11 years**
- The gene *STK11* on chromosome 19 has been found in a proportion of these patients.
- Characterized by **multiple GI hamartomatous polyps** (Fig. 18.31A) and **mucocutaneous** (mouth, buccal mucosa and genitalia region) **hyperpigmentation** (Fig. 18.31B).
- Associated with an **increased risk of several malignancies** and include cancers of the colon, pancreas, breast, lung, ovaries, uterus and testicles.

MORPHOLOGY

- **Site:** Most common in the **small intestine**.
- **Number:** Intestinal polyps are usually present in the **dozens**.
- **Size:** **Varies** from a few millimeters to several centimeters in diameter.
- **Gross:** **Large and pedunculated with a lobulated contour**.
- **Microscopy:**
 - Consists of **hyperplastic mature epithelium** appropriate to the anatomic site (where it develops) and **divided by broad bands of mature smooth muscle**.
 - Small intestinal PJ polyps consist of crypts and villi of varying lengths divided by **characteristic arborizing bands of smooth muscle fibers** (commonly fan out from the center of the polyp and has tree-like appearance) **intermixed with lamina propria** (Fig. 18.31C).

Peutz Jeghers polyp associated with:

- Mucocutaneous pigmentation
- Extragastrintestinal cancers.

PJ polyp (PJP): Most common in small intestine.

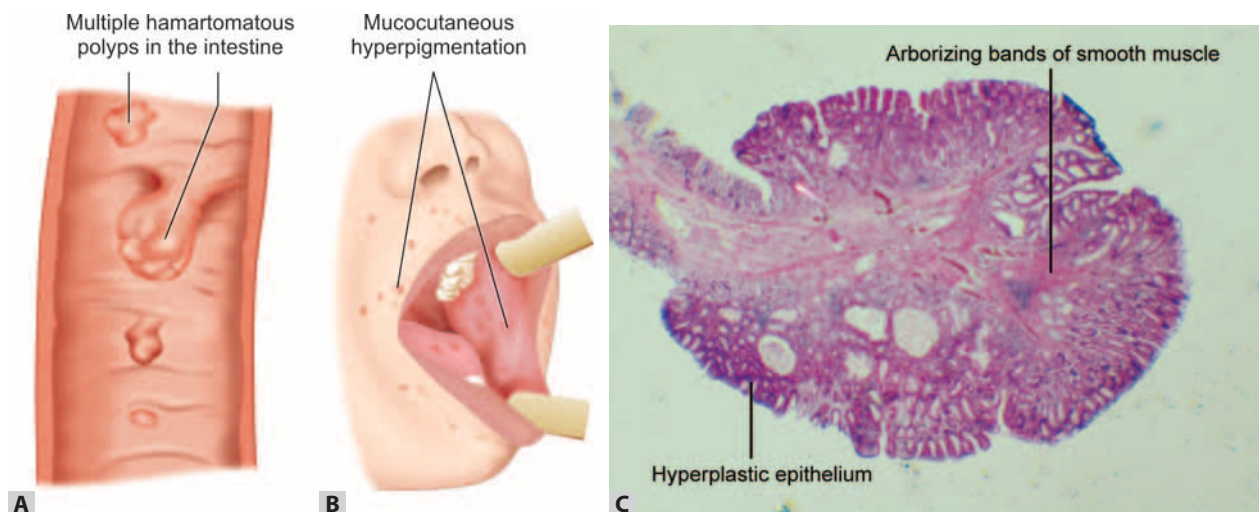
PJP: Increased risk of cancers of colon, breast and gynecologic cancers.

PJ polyps: Hamartomatous polyps likened to trees in which the trunk and branches are smooth muscle fibers.

Hyperplastic Polyps

Hyperplastic polyps: Most common non-neoplastic polyps of colon.

- Due to metaplastic proliferation of differentiated colonic epithelium



Figs 18.31A to C: (A) Multiple hamartomatous polyps in the intestine; (B) Mucocutaneous hyperpigmentation; (C) Whole-mount view of PJ polyp showing arborizing bands of mature smooth muscle lined by hyperplastic epithelium

- No malignant potential
- Most common non-neoplastic polyps of the colon and are frequently seen in the rectum
- Age: Sixth and seventh decades of life.

MORPHOLOGY

- **Gross**
 - **Site:** It is most common in the left colon.
 - **Size:** Small, smooth, sessile, nodular protrusions of the mucosa, less than 5 mm in diameter.
 - **Number:** Single or multiple.
- **Microscopy:** Composed of **elongated colonic crypts** lined by epithelial cells with a pseudopapillary configuration → “**saw-toothed**” or **serrated appearance**.

Neoplastic Polyps

Neoplastic polyp: Most common is adenoma.

- The most **common and important** neoplastic polyps are **colonic adenomas** (benign neoplasms of intestinal epithelium) with the **potential for transformation to colorectal adenocarcinomas**.
- Polyps can occur singly, synchronously in few numbers or as part of a familial polyposis syndrome.

Classification of Neoplastic Polyps

They are classified depending on:

1. **Growth pattern:** (a) Pedunculated, (b) sessile, or (c) flat or depressed. Pedunculated adenomas have thin fibromuscular stalks containing prominent blood vessels derived from the submucosa.
2. **Architecture:** Adenomas can be classified as **tubular**, **tubulovillous**, or **villous**.

Risk factors for malignant change in polyps of colon:

1. Large size (over 2 cm)
2. Villous architecture
3. Dysplasia
4. Multiple polyps.

MORPHOLOGY

General Features

- **Size:** Range from 0.3 to 10 cm in diameter.
- **Site:** **Almost 50%** all adenomatous polyps of the colon are located in the **rectosigmoid region**. The remaining 50% are evenly distributed throughout the rest of the colon.
- **Microscopy:** All **colorectal adenomas** are **low-grade dysplastic lesions** characterized by the presence of epithelial dysplasia. The epithelial dysplasia may be classified as mild, moderate and severe dysplasia.
- **Classification:** Depending on the architecture, adenomas can be classified as tubular, **tubulovillous**, or **villous**.

Tubular Adenomas (Adenomatous Polyps)

Q. List the differences between villous adenoma and tubulovillous adenoma.

They constitute two-third of the adenomas of large intestine.

- **Gross:** Appear as **small, smooth-surfaced, pedunculated polyps** usually **less than 2 cm** in diameter (Figs 18.32A and B). Tubular adenoma over 2 cm have higher risk of invasive carcinoma.
- **Microscopy:** Consists of **closely packed small rounded or tubular glands** (Fig. 18.32C) embedded in a **stroma** (increase in the number of glands and cells per unit area compared to the normal mucosa). The cells lining the glands are crowded and contain enlarged hyperchromatic nuclei. May show variable degree of epithelial dysplasia.

Tubular adenoma: Most common neoplastic polyps in the GI tract.

Villous Adenomas (villous Papilloma)

Q. Write short note on villous adenoma.

- They constitute one tenth of colonic adenomas and are **predominantly found in the rectosigmoid region**.
- **Gross:** **Large, broad-based, sessile**, elevated lesions with **cauliflower-like surface** (Figs 18.33A and B). **Most are over 2 cm**, but may be as large as 10–15 cm in diameter.
- **Microscopy:** Composed of **thin, long, finger-like projections**, (papillary, crown-like growth) which superficially **resemble the villi of the small intestine** (Fig. 18.33C). The lining epithelium shows dysplasia similar to tubular adenomas. However, **villous adenomas** (larger than 2 cm) likely **contain foci of carcinoma more commonly than tubular adenomas**.

Villous adenoma: Increased risk of developing carcinoma.

Tubulovillous Adenomas

Tubular adenoma: Like a raspberry on a stalk.

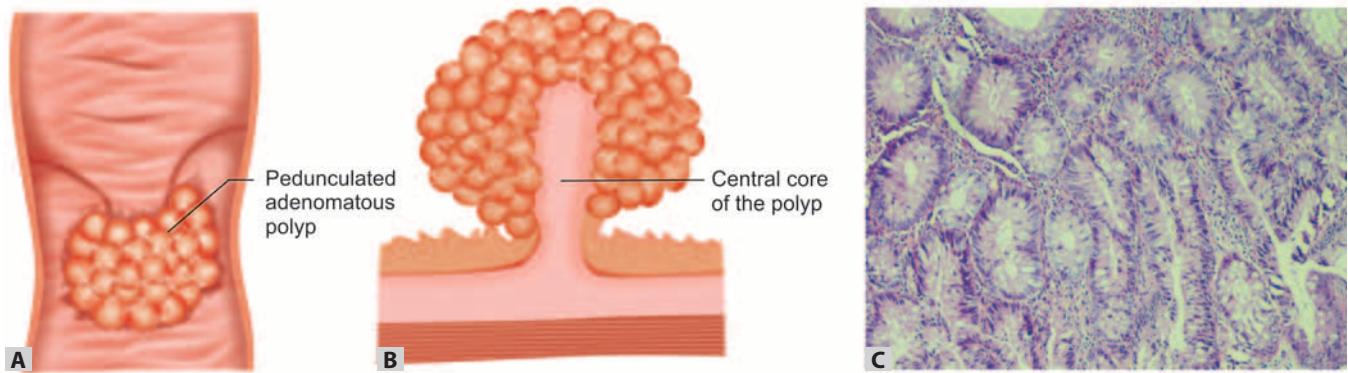
- They show a **mixture of both tubular and villous elements**. **Polyps with more than 25% and less than 75% villous component** are called as tubulovillous.

Sessile Serrated Adenomas

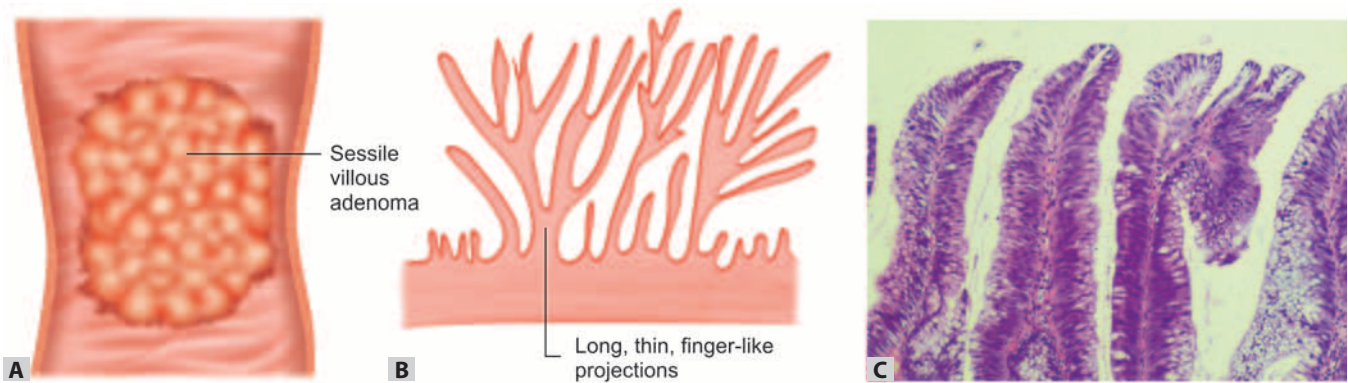
They are more common in the right colon and histologically overlap with hyperplastic polyps. They have malignant potential, but does not show dysplasia. Microscopically, consists of serrated architecture throughout the full length of the glands, including the base of the crypt, dilatation of crypt and lateral growth.

Intramucosal Carcinoma

It is term used when dysplastic epithelial cells break the basement membrane and invade into the lamina propria or muscularis mucosae. Since there are no functional



Figs 18.32A to C: Tubular adenoma: (A) Shows a pedunculated tubular adenomatous polyp (diagrammatic); (B) Cut section of tubular adenoma shows a central core (diagrammatic); (C) Photomicrograph shows closely packed tubular glands with dysplasia of lining epithelium



Figs 18.33A to C: Villous adenoma: (A) and (B) diagrammatic; (C) Thin, long, fingerlike projections covered by dysplastic epithelium

lymphatic channels in the colonic mucosa, intramucosal carcinomas do not have metastatic potential and complete polypectomy is usually curative.

Polyposis Syndromes

Q. Write short note on familial adenomatous polyposis.

- Polyps in the gastrointestinal system can develop either as sporadic lesions or as part of a polyposis or hereditary cancer syndrome.
- Several syndromes are characterized by the presence of colonic polyps and increased rates of colonic cancer.
- The most common syndromes include familial adenomatous polyposis and hereditary nonpolyposis colorectal cancer syndrome.

Familial Adenomatous Polyposis

- **Definition:** Familial adenomatous polyposis (FAP) is an autosomal dominant inherited syndrome characterized by hundreds to thousands of adenomas throughout

the colorectum as well as a numerous **extracolonic manifestations**.

- Patients develop few adenomas by the age of 21.

FAP: Caused by APC mutations and patients have more than 100 adenomas and 100% develop colonic cancer if untreated.

- **Genetic features:** It is caused by **germ-line** inactivation (5q) mutations of the **adenomatous polyposis coli (APC) gene**. APC is a tumor suppressor gene and its products modulate a specific signaling cascade (WNT signaling) that regulates cell proliferation (refer Fig. 18.35). Every cell of FAP patient has one inactive APC allele and inactivation of second allele initiate the early phase of neoplastic change.
- **Minimum of 100 polyps are necessary for a diagnosis of FAP.**
- **Morphology:** It similar to sporadic adenomas.

All untreated FAP patients develop colorectal adenocarcinoma, often before age 30. Prophylactic colectomy prevents colorectal cancer.

- **Gardner syndrome:** When the **extraintestinal manifestations of FAP are prominent**, the condition is known as Gardner syndrome. The extraintestinal lesions include **osteomas of mandible, skull, and long bones, epidermal cysts, desmoid tumors in the abdominal wall, thyroid tumors, etc.**
- **Turcot syndrome:** It is rare syndrome characterized by **colonic adenomas with tumors of the central nervous system** (particularly medulloblastomas).

Adenomatous polyposis coli gene (APC): On the short arm of chromosome 5.

Familial polyposis: Highest malignant potential.

Gardner syndrome: Colonic adenomas, epidermoid cysts, osteomas and desmoids.

Turcot syndrome: Colonic adenomas with brain tumors.

HNPCC: Caused by mutations (inactivation) in DNA mismatch repair genes.

Hereditary Non-polyposis Colorectal Cancer (HNPCC)/Lynch Syndrome

- HNPCC is the **colon cancer family syndrome not classically associated with large numbers of colonic adenomas.**
- **Despite the name hereditary nonpolyposis** colorectal cancer, **adenomas do develop** (though not in large numbers) in HNPCC patients.
- Transmitted as an **autosomal dominant** condition.
- HNPCC syndrome is characterized by increased risk of:
 - **Colorectal cancer:** HNPCC patients develop colonic cancer at **younger ages** than sporadic type and are often found in the **right proximal colon**. HNPCC-associated carcinoma are relatively nonaggressive, despite being poorly differentiated and mucinous type.
 - **Extra-colonic cancers:** These include **cancers of the endometrium, ovary, stomach and small intestines, and urinary bladder.**
- **Genetics of HNPCC:** Caused by **germ-line mutations in DNA mismatch repair (MMR) genes**. These genes **encode proteins involved in the detection, excision and repair of errors that occur during replication of DNA.**
 - **One result of defective DNA MMR gene** is a phenomenon referred to as **microsatellite instability (MSI)**. MSI occurs in about 90% cancers in HNPCC.
 - Appearance of abnormally long (due to increase in the number of nucleotide repeats) or short (due to decrease in the number of nucleotide repeats) microsatellites in a DNA (in normal tissue versus tumor) is referred to as **microsatellite instability.**

- MSI is divided into three groups depending on the alterations in microsatellite length: MSI-High (MSI-H), MSI-Low (MSI-L), and MS-Stable (MS-S).
- About six **mismatch repair (MMR) genes** have been identified which are designated as *hMSH2*, *hMSH6*, *hMLH1*, *hMLH3*, *hPMS1* and *hPMS2*, but the majority of HNPCC cases involve two genes namely **MSH2 and MLH1.**
- The MMR genes act as tumor suppressors and loss of both copies of the genes result in unrestrained growth and ultimately neoplastic transformation.
- HNPCC patients inherit one defective copy of an MMR gene, and the second copy of MMR gene is lost or inactivated as a somatic event. Loss of the second MMR gene often occurs due to methylation of the gene promoter.

Microsatellites represent short (1–6 nucleotides) repetitive DNA sequences (tandem repeats) scattered throughout the genome.

COLORECTAL CANCER: ADENOCARCINOMA

Adenocarcinoma of the colon (colorectal carcinoma) is the **most common malignant tumor of the GI tract.**

Cecum: Widest portion of colon.

Sigmoid colon: Narrowest portion of colon.

Etiology

Q. Write short note on etiopathogenesis of carcinoma of colon.

The rate of colorectal cancer has increased significantly, probably as a result of changes in lifestyle and diet.

Dietary Factors

Closely associated with increased colorectal cancer rates.

- **Low intake of dietary fiber:** It is associated with decreased stool bulk → leads to **slower transit of fecal contents** through the colon and altered composition of the intestinal microbiota. These changes may **increase synthesis of toxic oxidative by-products** of bacterial metabolism, which **remains in contact with the colonic mucosa for longer periods.** The dietary fiber may bind to potential mutagens and dilute their concentration by increasing stool bulk.
- **Dietary high intake of refined carbohydrates and fat:** High level of animal fat (found in red meats and processed meat) in the diet is associated with **increased incidence of colorectal cancer.** High fat intake increases the

hepatic synthesis and secretion of bile into the intestine. The contents present in the bile such as cholesterol and bile acids can be converted into carcinogens by intestinal bacteria.

- **Other dietary factors:** Diets rich in cruciferous vegetables (e.g. cauliflower, Brussels sprouts and cabbage) and **vitamin A** may be associated with a **lower incidence of colorectal cancer**. Deficiencies of vitamins A, C and E, which act as antioxidants (free-radical scavengers) may increase the damage caused by oxidants.

Dietary factors in colorectal cancer:

Low intake of dietary fiber

High intake of refined carbohydrates and fat

Deficiency of vitamins A, C and E.

Protective Effect of Aspirin or Other NSAIDs

- Ingestion of therapeutic agents such as aspirin or other nonsteroidal anti-inflammatory drugs (NSAIDs) may reduce the risk of colorectal cancer.
- It may be due to these agents causing **inhibition of the enzyme cyclooxygenase-2 (COX-2)**, which is highly expressed in 90% of colorectal carcinomas and 40–90% of adenomas.
- COX-2 is required for production of prostaglandin E₂, which promotes epithelial cell proliferation, particularly after injury.

Adenoma-carcinoma Sequence

The colonic adenocarcinoma may evolve from the pre-existing adenomas, referred to as adenoma-carcinoma sequence discussed below under pathogenesis.

Hereditary Non-polyposis Colorectal Cancer (HNPCC)

Colon cancers in HNPCC patients develop at **younger ages** than sporadic colon cancers and are often found in the **right colon** (refer page 516).

Risk Factors

- **Increasing age:** It is the most important risk factor. Risk is low before age 40 and it increases steadily to age 50, after which it doubles with each decade.
- **Family history of colonic cancer** in first degree relative.
- **Prior colorectal cancer:** It increases the risk for a subsequent tumor.
- **Ulcerative colitis and Crohn disease:** They have increased risk of colorectal cancer.
- **Others:** Physical inactivity, obesity (body and abdominal), smoking, alcohol excess (especially beer) and sugar consumption are some of the other risk factors.

Colon cancer: Third most common cancer in men and women.

Pathogenesis

Most colorectal cancers develop from the combination of multiple molecular alterations. They can be mainly divided into 1) **genetic abnormalities** (that **activate oncogenes** or **inactivate tumor suppressor genes**) and 2) **epigenetic abnormalities**.

Genetic Pathways

Two distinct genetic pathways have been described: A) Classic adenoma-carcinoma sequence B) microsatellite instability pathway.

A. Adenoma-carcinoma Sequence (APC/β-catenin Pathway)

Q. Write short note on adenoma-carcinoma sequences in colonic carcinoma.

Seen in **about 80% of sporadic colonic cancers**. According to this pathway (Fig. 18.34), the **morphologic progression in a specific step-wise sequence from normal mucosa to adenoma to invasive cancer** is accompanied by a **series of multiple molecular alterations**.

1. **Inactivation of APC tumor suppressor gene** (Fig. 18.35)
 - APC is an important **negative regulator of β-catenin**, a component of the **WNT signaling pathway**. Normally, the product of APC gene (APC protein) binds to and causes degradation of β-catenin and prevents proliferation of cells.
 - **Both copies of APC gene** must be **inactivated** either by mutation or epigenetic (methylation) events. This leads to **accumulation of β-catenin**, which forms a complex with TCF → activates transcription of *c-MYC*, *cyclin D1*, and other genes → **promotes cell proliferation**. One of the syndrome associated with APC inactivation is familial adenomatous polyposis (refer pages 515).
2. **K-RAS mutations:** Loss of APC function is followed by mutations in proto-oncogene *K-RAS*, which results in oncogene *K-RAS* → **promotes cell proliferation and also prevents apoptosis**. *K-RAS* mutation is a late event.
3. **SMAD2 and SMAD4 (tumor suppressor gene) mutations:** These may develop as the neoplasm progresses. These genes are effectors of TGF-β signaling, which is normally a potent inhibitor of cell proliferation. The loss of these tumor suppressor genes may lead to uncontrolled cell proliferation.
4. **Mutation of TP53 (tumor-suppressor gene):** It is seen in **70–80% of colon cancers**, but is not commonly affected

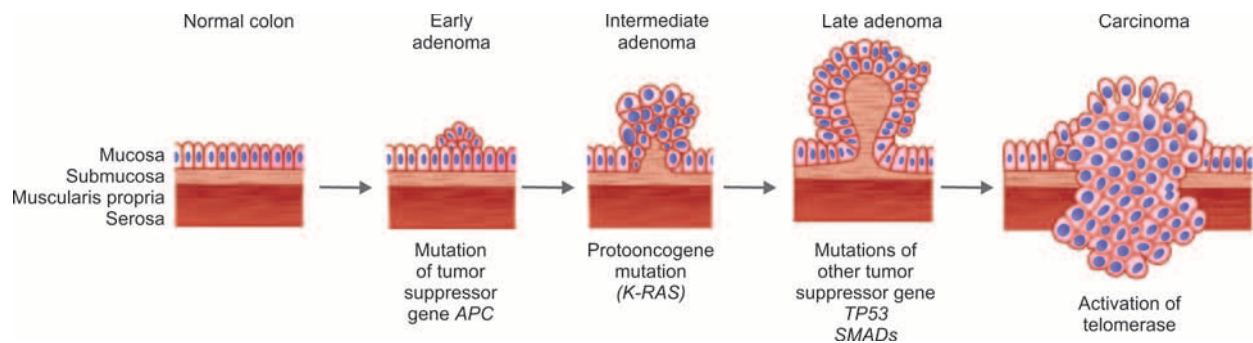
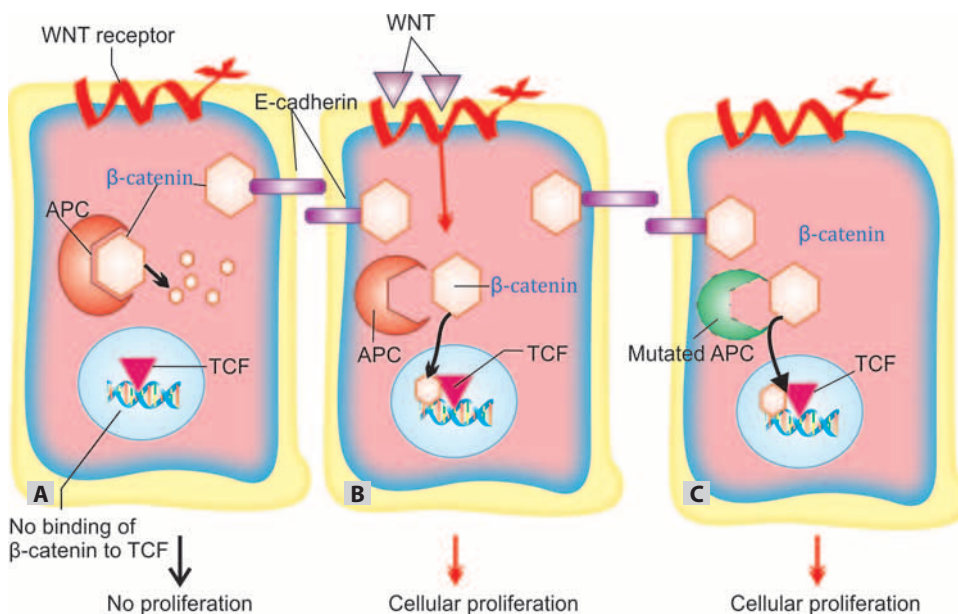


Fig. 18.34: Adenoma-carcinoma sequence showing morphological and molecular changes. Loss of tumor suppressor gene *APC* occurs early followed by mutations of *K-RAS*, *SMADs* and inactivation of the tumor suppressor gene *TP53*, and activation of telomerase



Colon cancer: Third most common cause of death due to cancer.

APC: Participates in cell cycle control by regulating the intracytoplasmic pool of β -catenin.

APC: Influences cell cycle proliferation by regulating WNT expression.

APC gene:

- Tumor suppressor gene on chromosome 5q21
- Its product forms a cytoplasmic complex with GSK-3 β (a serine-threonine kinase), β -catenin and axin.

Figs 18.35A to C: Role of APC in regulating the β -catenin. APC and β -catenin are components of the WNT signaling pathway: (A) In resting cells, β -catenin forms a complex with APC protein. This complex destroys β -catenin; (B) When cells are stimulated by WNT molecules, the β -catenin is not destroyed and its cytoplasmic levels increase. β -catenin translocates to the nucleus, where it binds to TCF, a transcription factor that activates cell cycle progression; (C) When *APC* is mutated or absent, the β -catenin cannot be destroyed and cell enters into the cell cycle

in adenomas. *TP53* mutations also occur at late stages of tumor progression. **Mechanism of inactivation of tumor suppressor gene:**

- **Chromosomal deletions**
- **Methylation of a CpG-rich zone, or CpG island** (a region of some genes that frequently includes the promoter and transcriptional start site). Cytosines in CpG dinucleotides are normally unmethylated. Methylating the cytosine can turn the gene off (**gene silencing**).

"CpG" is shorthand for "C—phosphate—G", that is, cytosine and guanine separated by only one phosphate.

5. **Activation of telomerase:** Telomere plays a role in stabilizing the chromosome. It shortens with each cell division until cell senescence develops (refer pages 33 and 195).

- Telomerase is required to maintain telomere stability and hence cell immortality.
- Most adenomas do not show telomerase activity, but colorectal carcinoma have increased telomerase activity.
- **Expression of telomerase** also increases as lesions progress.

Adenoma-carcinoma sequence showing morphological and molecular changes are shown in Figure 18.34.

Microsatellite Instability Pathway (Defective DNA Repair) (Fig. 18.36)

Microsatellites: Repeated sequences (tandem repeats) of one to six (1–6) nucleotides in the genome.

Microsatellites are repeated sequences (tandem repeats) of one to six (1–6) nucleotides in the genome. They become **unstable during normal cellular replication**, leading to insertion or deletion of bases within these regions. **DNA mismatch repair (MMR) genes rapidly correct these errors to maintain microsatellite length.**

- **Mutations in normal DNA repair genes (mismatch-repair defects) leads to accumulation microsatellites** → referred to as **microsatellite instability (MSI)**. About six **mismatch repair (MMR) genes** have been identified, which are designated as *hMSH2*, *hMSH6*, *hMLH1*, *hMLH3*, *hPMS1* and *hPMS2* (refer HNPCC in page 516).
- Microsatellites are typically formed in noncoding regions. However, some microsatellite sequences are located in the coding or promoter region of genes involved in regulation of cell growth. Example, **microsatellite instability** involving:
 - **Type II TGF- β receptor:** Normally, TGF- β inhibits colonic epithelial cell proliferation and mutation in type II TGF- β receptor can lead to uncontrolled cell growth.
 - **Pro-apoptotic gene *BAX*:** Its mutation can lead to loss of *BAX*, which may increase the survival of genetically abnormal clones of cells.

- DNA mismatch repair defects may also lead to **mutations in the oncogene *BRAF*** and silencing of groups of genes (such as *MLH1*) due to CpG island hypermethylation.
- Defective DNA repair gene can lead to combination of microsatellite instability, *BRAF* mutation, and methylation of specific targets (*MLH1*).

Microsatellites become unstable during normal cellular replication, leading to insertion or deletion of bases within this region.

Microsatellite instability (MSI): Accumulation of microsatellites.

Defects in MMR genes:

- Microsatellite instability
- Mutations in many genes involved in cell proliferation, differentiation and/or apoptosis.

Epigenetic Abnormalities (refer page 182-183)

Definition: Epigenetics is a **reversible, heritable** alteration in gene expression, which occurs without mutation.

- It is unrelated to gene nucleotide sequence.
- Epigenetic events may enhance progression along both genetic pathways mentioned above.
- Epigenetic changes involve histone modification and DNA methylation, both of which affect gene expression.
- In **carcinoma colon cells silencing DNA repair genes (mismatch-repair gene *MLH1*) by hypermethylation** can occur.

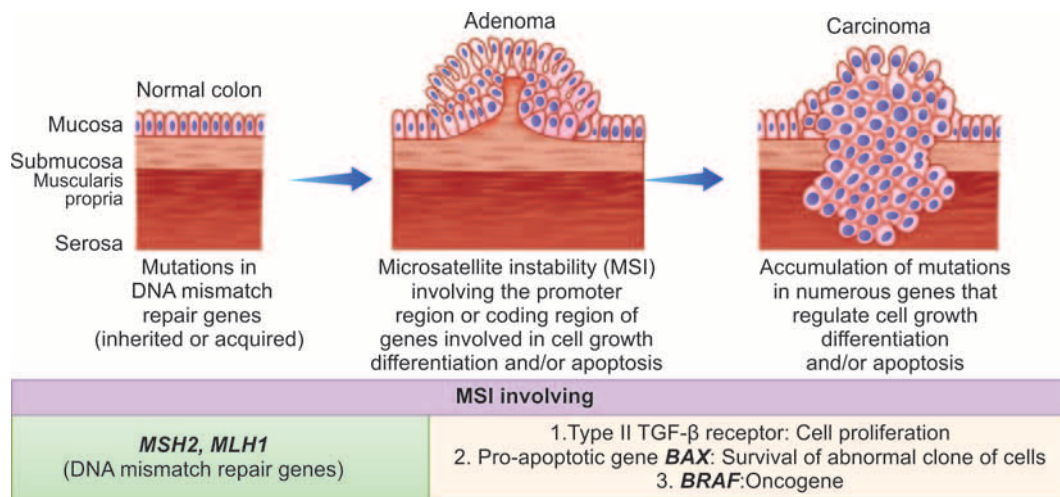


Fig. 18.36: Morphological and molecular changes in the microsatellite instability pathway of colon carcinogenesis. Defects in mismatch repair genes result in microsatellite instability and allows accumulation of mutations in numerous genes involved in cell survival and proliferation

MORPHOLOGY**Q. Write short note on morphology of carcinoma colon.****Gross**

Location: Colonic adenocarcinomas can be found in **any location** of the colon.

Types: Grossly colonic carcinomas can be categorized into four general types:

- **Exophytic polypoid mass in the right-side of colon** (Fig. 18.37A): Tumors in the proximal colon usually grow into the lumen as bulky, **exophytic (cauliflower-like), polypoid**, and masses and extend along one wall of the cecum and ascending colon. They rarely cause intestinal obstruction.
- **Annular and constricting tumors in the left-side of colon** (Fig. 18.37B): These tumors are annular lesions that produce the characteristic “napkin-ring” or “apple core” constrictions and luminal narrowing. It **may be associated with intestinal obstruction** and dilatation with attenuation and flattening of the mucosal folds of colon proximal to the tumor. The tumors are firm due to associated desmoplasia.
- **Diffuse/tubular tumors** (Fig. 18.37C): These are similar to linitis plastica of the stomach. They show diffuse flattening and **thickening of the colon**, initially involving the mucosa, but later involve the entire wall of intestine.
- **Infiltrative and ulcerating tumors** (Fig. 18.37D): These cancers are **usually raised, have irregular edges** and a **central, excavated ulcerated area** that often infiltrate the deep layers of the bowel wall.

Microscopy (Fig. 18.38)

- Majority of colonic cancers are **adenocarcinomas** and are similar on microscopic examination. Adenocarcinoma may be **well-differentiated, moderately or poorly differentiated**.
- Most of the tumors show **glands of variable size and configuration** separated by moderate amount of stroma. Mitotic figures are usually abundant. The **lumen** of the glands is usually **filled with inspissated eosinophilic mucus and nuclear and cellular debris (“dirty” necrosis)**. The invasive component of these tumors may show stromal desmoplasia → causes firm consistency.
- Poorly differentiated carcinoma shows only few glands.
- **Mucinous adenocarcinomas:** They secrete abundant mucin and accumulate within the intestinal wall and are associated with poor prognosis.
- **Signet-ring carcinoma:** It consists of **signet-ring cells** similar to those in gastric cancer and constitute more than 50% of the tumors.

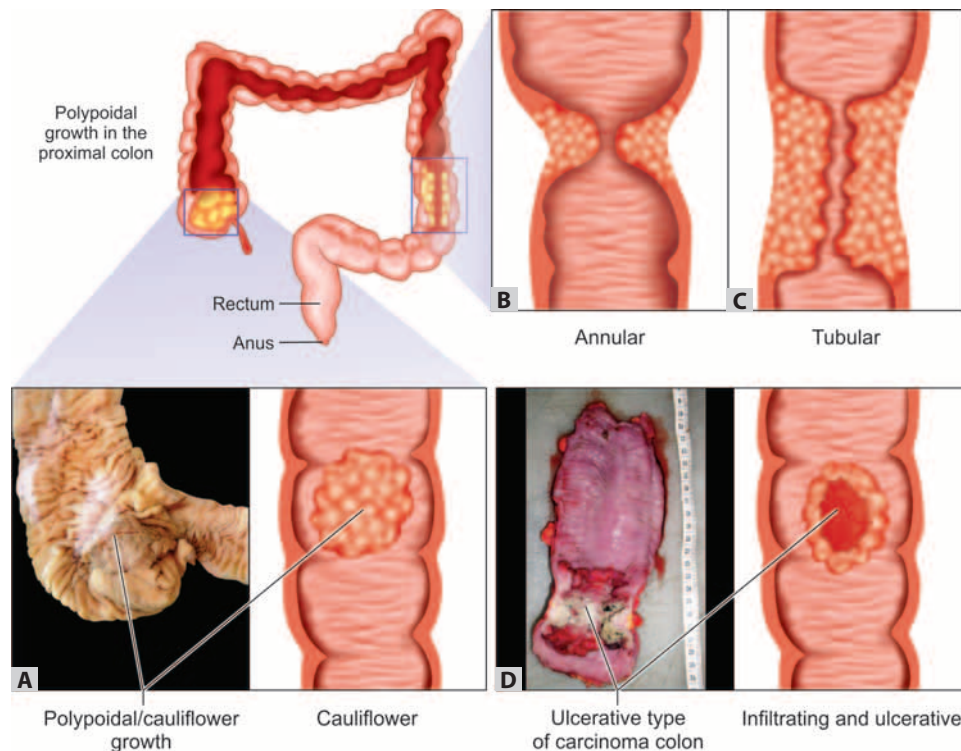
Carcinoma colon:

- Exophytic polypoid mass in the right colon
- Annular constricting mass in the left colon.

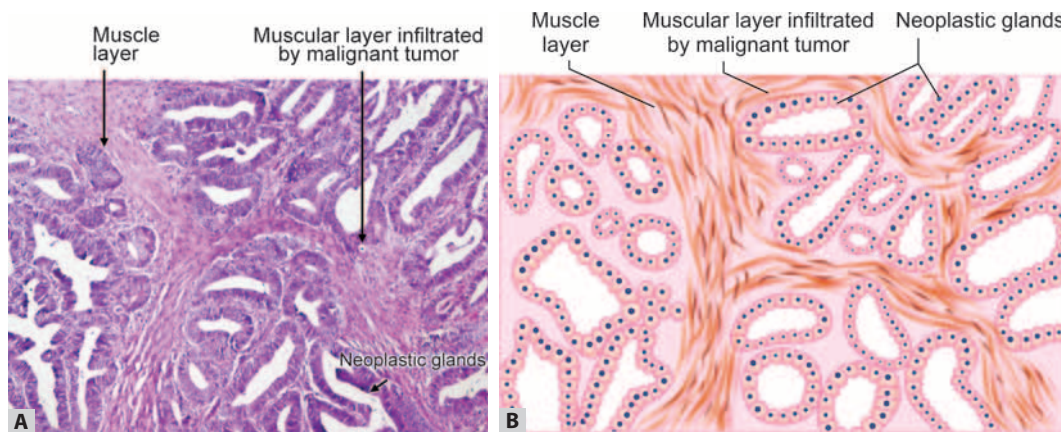
Most common site of colon cancer is sigmoid colon.

Least common site of colonic cancer is: Hepatic flexure.

Colon cancer: Mucin production worsens the prognosis since mucin aids in tumor extension.



Figs 18.37A to D: The four common macroscopic varieties of carcinoma of the colon: (A) Exophytic/cauliflower/polypoid (left side- gross specimen); (B) Annular; (C) Tubular; and (D) Infiltrating and ulcerative (left side- gross specimen). Carcinoma of proximal colon are usually polypoid and exophytic. Carcinoma of distal colon are usually annular



Figs 18.38A and B: Adenocarcinoma of colon composed of tumor cells arranged in glandular pattern: (A) Photomicrograph; (B) Diagrammatic

Clinical Features

Q. Differences between the carcinoma of colon on right and left side.

- Tumors in the cecum and other **right-sided colon** cancers usually present with **fatigue and weakness** due to **iron deficiency anemia**. Thus, it is important that **iron deficiency anemia** in an older man or postmenopausal woman should be considered as due to GI cancer until otherwise proved.
- **Left-sided cancers** may produce **occult bleeding, altered bowel habits or pain and discomfort** in the left lower quadrant.

Methods of Investigation of Colon Cancer

1. **Occult blood** loss in the stool by Guaiac test.
2. **Tumor markers:** Elevated levels of **carcinoembryonic antigen (CEA)** and **CA 19-9**.
3. Flexible sigmoidoscopy.
4. **Colonoscopy** helps in **direct visualization of cancer** and may be used to **take a biopsy**: Investigation of choice.
5. **Radiology:**
 - **Double-contrast barium enema:** It is the radiological investigation of choice, when colonoscopy is contraindicated. It characteristically shows “**apple core**” appearance.
 - **Ultrasonography:** Used as a screening investigation for liver metastases.
 - **Spiral CT:** Elderly patients when contrast enemas or colonoscopy are not diagnostic or are contraindicated.

Staging and Prognosis

Colonic cancer: Most important prognostic factors—

1. Depth of invasion
2. Lymph node status.

- **Two most important prognostic factors are depth of invasion and the presence or absence of lymph node metastases.**
- Invasion into the muscularis propria reduces the survival rate which is reduced further in the presence of lymph node metastases.
- Poorly differentiated and mucinous carcinomas are associated with poor prognosis.

Dukes and Kirklin, and Astler-Collier staging were used being presently replaced by **TNM (tumor-nodes-metastasis) classification** and staging system from the American Joint Committee on Cancer.

Carcinoembryonic antigen (CEA) may be elevated in colonic cancer.

Spread

The tumour can spread in a longitudinal, transverse or radial direction; it spreads round the intestinal wall and usually causes intestinal obstruction before it invades adjacent structures.

- **Direct spread:** The tumor can spread in a transverse, longitudinal, or radial direction.
 - Transverse spread circumferentially round the intestinal wall causes intestinal obstruction.
 - Longitudinal spread along both directions.

- Radial spread directly into the submucosa into the muscularis propria and thence out into the serosa, pericolic fat, lymphatics and veins in the mesentery alongside the bowel wall and sometimes into the peritoneal cavity.
- **Lymphatic spread:** Tumor may spread through lymphatics into the regional lymph nodes. Lymph nodes draining the colon are grouped as:
 - N1: Nodes in the immediate vicinity of the bowel wall
 - N2: Nodes arranged along the ileocolic, right colic, midcolic, left colic and sigmoid arteries
 - N3: Apical nodes around the superior and inferior mesenteric vessels.
- **Blood spread: Venous invasion** may give rise to blood-borne metastases in the **liver** (through portal vein). It may also spread to **lungs and bones**.

Carcinoma colon: Most **common site of metastasis liver>lung**.

- **Transcoelomic spread:** Rarely, it can spread by dislodging tumor cells from the serosa of the bowel or

via the subperitoneal lymphatics to other structures within the peritoneal cavity.

ACUTE APPENDICITIS

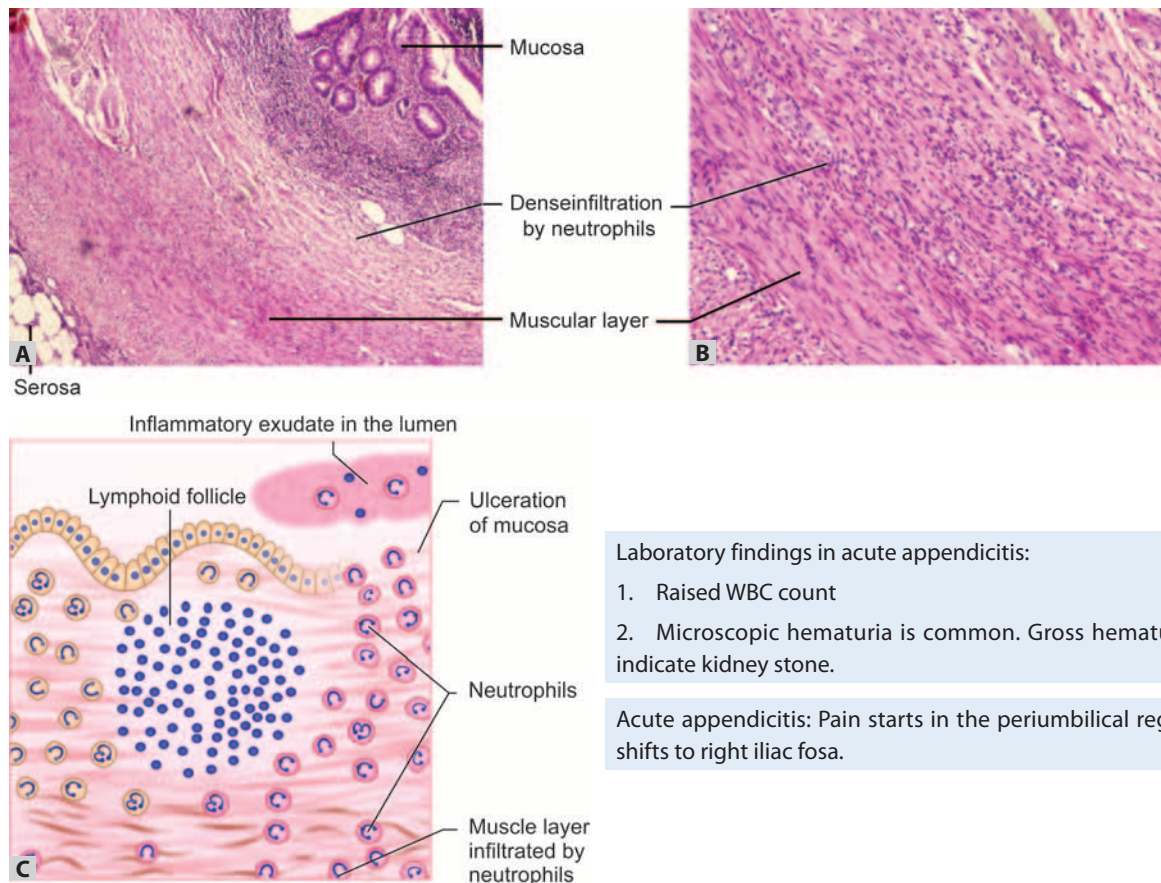
Acute appendicitis: Common abdominal surgical emergency.

- The appendix is prone to acute and chronic inflammation.
- Acute appendicitis is an acute inflammatory process involving the appendix.
- Acute appendicitis can occur in any age group but is most common in adolescents and young adults.

Worldwide, perforated appendicitis is the leading general surgical cause of death.

Pathogenesis

- The etiology of appendicitis is multifactorial and may involve obstruction, ischemia, infections and hereditary factors.



Laboratory findings in acute appendicitis:

1. Raised WBC count
2. Microscopic hematuria is common. Gross hematuria may indicate kidney stone.

Acute appendicitis: Pain starts in the periumbilical region and shifts to right iliac fossa.

Figs 18.39A to C: (A) Photomicrograph (low power) of acute appendicitis showing inflammation of mucosa, submucosa and muscular layer; (B) (High power view) shows numerous neutrophils in the muscular layer; (C) Diagrammatic appearance of acute appendicitis

- Acute appendicitis is thought to begin with luminal obstruction due to the factors, which progressively increases the intraluminal pressure.
- The obstruction is usually caused by small stone-like mass of stool, or *fecalith* or less commonly, a gallstone, tumor, or mass of worms (*Oxyuriasis vermicularis*).
- Ischemic injury and stasis of luminal contents favor bacterial proliferation, trigger inflammatory response.
- Inflammation produces edema and neutrophilic infiltration of the lumen, muscular wall and periappendiceal soft tissues.
- The pressure produced by inflammation and edema predisposes to the development of gangrene, perforation, and peritonitis.

Acute appendicitis: Most common bacteria isolated in perforated appendicitis is: *Bacteroides fragilis* (80%) > *E. coli* (77%).

MORPHOLOGY

Gross

- The appendix may be **swollen and erythematous**
- The **serosa** initially appears dull and gray and later may be **covered by a purulent exudate**
- Perforation secondary to gangrene can follow and form abscess.

Microscopy (Fig. 18.39)

- The early lesions show **mucosal erosions**.
- Later, the inflammation extends into the lamina propria, and collections of neutrophils may also be seen in the lumen of the appendix.
- Diagnosis of acute appendicitis should be made when **muscularis propria shows infiltration by neutrophils**.
- In severe cases, neutrophilic exudate produces fibrinopurulent reaction in the serosa. When focal abscesses develop within the wall, it is termed as **acute suppurative appendicitis**.
- When appendix shows large areas of hemorrhagic ulceration and gangrenous necrosis, it is known as **acute gangrenous appendicitis**. This may rupture leading to suppurative peritonitis.

Clinical Features

Acute appendicitis presents with pain in the periumbilical region, which ultimately localizes to the right lower quadrant. This is followed by nausea, vomiting, low-grade fever and mild leukocytosis.

McBurney's sign: A classic physical finding is deep tenderness located two-thirds of the distance from the umbilicus to the right anterior superior iliac spine (McBurney's point).

Complications: Gangrenous appendicitis, perforation, pyelophlebitis, portal venous thrombosis, liver abscess, and bacteremia.

Hepatobiliary Disorders

LIVER

BILIRUBIN METABOLISM AND BILE FORMATION (FIG. 19.1)

Q. Write short note on bilirubin metabolism.

Bilirubin Production

Source of Bilirubin

Unconjugated bilirubin: End product of heme degradation.

- **Major** (85%) is derived from the **catabolism of hemoglobin** during the breakdown of senescent red cells.
- **Minor** (15–20%) is derived from the degradation of heme produced from **other sources** (e.g. the P-450 cytochromes) and from premature destruction of hemoglobin in developing red cell precursors in the bone marrow.

Bilirubin Formation

Unconjugated bilirubin:

- Lipid soluble and water insoluble
- Toxic to the brain in newborns.
- Heme liberated from above sources oxidized to **biliverdin** by heme oxygenase.
- **Biliverdin** is immediately **reduced to bilirubin** by the enzyme biliverdin reductase. The bilirubin formed is known as **unconjugated bilirubin**.

Transport of Bilirubin/Bilirubin Binding

- Unconjugated bilirubin formed in the periphery is liberated into the circulation → reversible **binding of bilirubin to serum albumin** (albumin–bilirubin complex). Unconjugated bilirubin is insoluble in aqueous solutions at physiologic pH.
- The unconjugated bilirubin is **transported to the liver** in plasma.

Hepatic Processing of Bilirubin

Excretion of bilirubin from the body is one of the major function of the liver. Metabolism of bilirubin in the liver consists of four separate but interrelated events:

- **Hepatic uptake from the circulation:** On reaching the sinusoidal plasma membrane of the hepatocyte, the unconjugated bilirubin (albumin–bilirubin complex) is dissociated → enters the hepatocytes.
- **Binding:** Within the hepatocyte, bilirubin binds to several proteins in the cytosol known collectively as **glutathione-S-transferases** (also termed ligandin).
- **Conjugation with glucuronic acid:**
 - **Unconjugated bilirubin** (not water-soluble) combines with one or two molecules of glucuronic acid in the presence of uridine diphosphate (UDP)–glucuronyltransferase (UGT1A1) → forms **water-soluble bilirubin diglucuronide (conjugated bilirubin)**.
- **Biliary excretion/secretion:**
 - Conjugated bilirubin is excreted into bile → reaches the small intestine.

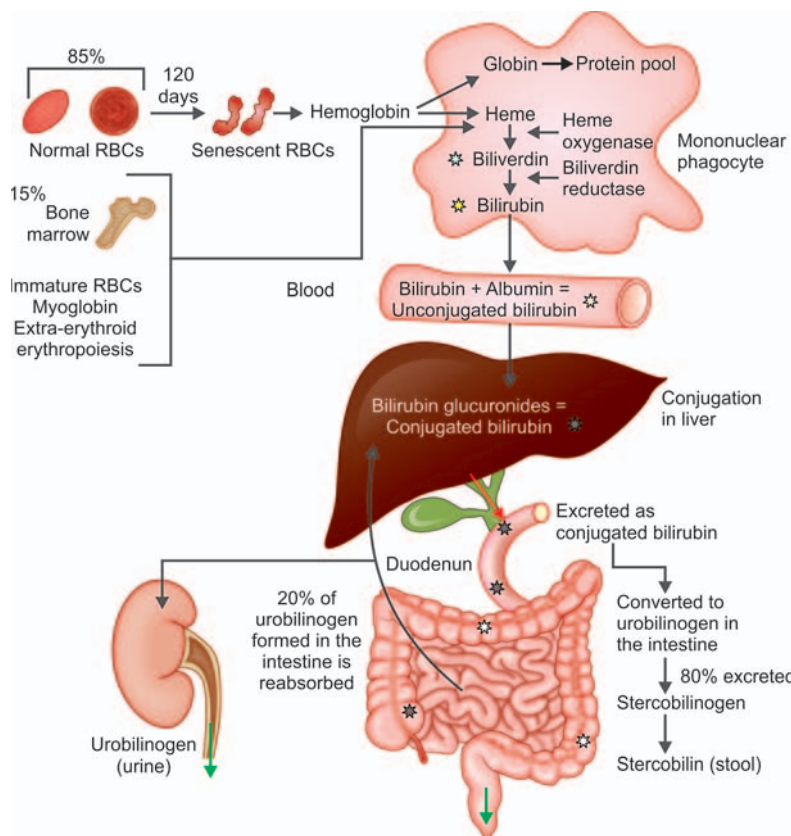


Fig. 19.1: Bilirubin metabolism. Major bilirubin is derived from the breakdown of senescent circulating RBCs. The bilirubin binds to serum albumin and delivered to the liver. In the liver, bilirubin forms conjugated water-soluble bilirubin and excreted into bile. In the gut, bacteria deconjugate the bilirubin and degrade it to colorless urobilinogens. About 80% urobilinogens excreted in the stool, and about 20% is reabsorbed. Minor fraction of reabsorbed is excreted into urine as urobilinogen

Intestinal Phase of Bilirubin Metabolism

Intestinal bacteria: Convert conjugated bilirubin to urobilinogen.

In the intestine, conjugated bilirubin has two fates:

1. **Most of the conjugated bilirubin deconjugated** (in the distal small intestine and colon) by bacterial β -glucuronidases \rightarrow to **colorless urobilinogens**. The urobilinogen has two fates:
 - i. **Excretion of urobilinogen:** **Most (80%)** of the urobilinogen is **excreted in the feces as stercobilinogen** (is responsible for the normal color of the stool).
 - ii. **Reabsorption of urobilinogen:**
 - ◆ About 20% of urobilinogens is reabsorbed in the terminal ileum and colon into portal circulation \rightarrow reaches the liver \rightarrow reexcreted into the bile.
 - ◆ A small amount of reabsorbed urobilinogen is excreted in the urine as urobilinogen.
- Note:** 1. In obstructive jaundice, urine does not contain urobilinogen. 2. In hepatocellular and

hemolytic jaundice, urinary urobilinogen is markedly increased (more than 4 mg/day).

2. Part of the conjugated bilirubin is excreted in the stool as such (bilirubin glucuronide).

Obstructive jaundice: Stercobilinogen is absent in the stool and stool appears pale and clay colored.

Stool: Urobilinogen \rightarrow stercobilinogen responsible for color of stool.

Cholestasis: Systemic retention of bilirubin and other solutes eliminated in bile.

Urobilinogen: ~20% recycled to liver and kidney.

Renal Excretion of Bilirubin

- Unconjugated bilirubin is tightly bound to albumin; cannot be filtered by the glomeruli and not excreted in the urine.

- Conjugated bilirubin is filtered by the glomeruli and appears in the urine (bilirubinuria).

JAUNDICE

Definition: Jaundice is defined as **yellowish pigmentation of skin, mucous membranes and sclera** due to **increased levels of bilirubin** in the blood. The scleral involvement is because it is rich elastic tissue that has special affinity for bilirubin.

- **Normal serum bilirubin level:** In the normal adult range from 0.3 to 1.2 mg/dL.
- **Jaundice:** Clinically detected when the serum bilirubin level is above 2.0–2.5 mg/dL. With severe disease, the levels may be as high as 30–40 mg/dL.

Normal bilirubin level: Balance between rate of bilirubin production and rate of biliary excretion.

Jaundice: Yellowish pigmentation of skin, mucous membranes and sclera due to increased levels of bilirubin.

Mechanism of Jaundice

Jaundice: Occurs when the equilibrium between bilirubin production and excretion is disturbed. Serum bilirubin level is above 2.0–2.5 mg/dL.

Various mechanisms can produce jaundice (Table 19.1). Generally, one of these mechanisms predominates. However, more than one mechanism may be responsible for jaundice.

TABLE 19.1: Various mechanisms of jaundice

Mechanism	Type of hyperbilirubinemia
Excessive extrahepatic production of bilirubin	Unconjugated hyperbilirubinemia
Reduced hepatocyte uptake	
Impaired conjugation	
Decreased hepatocellular excretion	Predominantly conjugated hyperbilirubinemia
Impaired bile flow	

During first 2 weeks of life the process of conjugation and excretion of bilirubin is not fully mature.

Both unconjugated bilirubin and conjugated bilirubin may accumulate in systemic circulation. There are two main pathophysiologic differences between the two forms of bilirubin.

Unconjugated bilirubin: Insoluble in water and cannot be excreted in urine.

- **Unconjugated bilirubin:**
 - **Insoluble in water** at physiologic pH and is present in the circulation forming tight complexes with serum albumin.
 - **Cannot be excreted in the urine** even when its levels are high in the blood.
 - In **hemolytic disease of the newborn** (erythroblastosis fetalis) → excessive unconjugated bilirubin → crosses the blood brain barrier → reach the brain → causes severe neurologic damage → referred to as **kernicterus**.

Kernicterus: Neurological damage produced in infants due to crossing of unconjugated bilirubin through immature blood brain barrier.

- **Conjugated bilirubin:**
 - **Water-soluble, nontoxic**, and only loosely bound to albumin in the plasma.
 - **Excess conjugated bilirubin in plasma** can be excreted in urine.

Classification of Jaundice

Other classification of jaundice: 1) Prehepatic, hepatic and post-hepatic and 2) Medical and surgical.

1. **Based on the underlying cause** (Box 19.1)
 - Predominantly unconjugated.
 - Predominantly conjugated.
2. **Based on pathological mechanism:**
 - Hemolytic jaundice.
 - Hepatocellular jaundice.
 - Obstructive jaundice.

HEREDITARY HYPERBILIRUBINEMIAS (TABLE 19.2)

Crigler–Najjar Syndrome

Q. Write short note on Crigler–Najjar syndrome.

Crigler–Najjar Syndrome Type I

Crigler–Najjar syndrome: Basic abnormality is impaired conjugation of bilirubin.

- Rare, **autosomal recessive** disorder due to **complete absence of hepatic UGT1A1**.

BOX 19.1: Classification of jaundice**A. Predominantly unconjugated hyperbilirubinemia**

1. Increased production of bilirubin
 - Hemolytic anemias
 - Resorption of blood from internal hemorrhage (e.g. GI bleeding, hematomas)
 - Ineffective erythropoiesis (e.g. pernicious anemia, thalassemia)
2. Reduced hepatic uptake
 - Drug that interfere with membrane carrier systems
 - Diffuse liver disease (hepatitis, cirrhosis)
 - Some cases of Gilbert syndrome
3. Impaired bilirubin conjugation
 - Physiologic jaundice of the newborn
 - Crigler–Najjar syndrome types I and II
 - Gilbert syndrome
 - Diffuse liver disease (e.g. hepatitis, cirrhosis)

B. Predominantly conjugated hyperbilirubinemia

1. Decreased hepatocellular excretion
 - Deficiency of canalicular membrane transporters
 - Dubin–Johnson syndrome
 - Rotor syndrome
 - Liver damage or toxicity (e.g. hepatitis)
2. Impaired intra/extrahepatic bile flow
 - Inflammatory destruction of bile ducts (e.g. primary biliary cirrhosis)
 - Gallstones
 - Carcinoma of pancreas

Neonatal jaundice or physiologic jaundice of the newborn: Transient and mild unconjugated hyperbilirubinemia.

Neonatal jaundice: May be exacerbated by breastfeeding, due to the presence of bilirubin-deconjugating enzymes in breast milk.

Jaundice: Viral hepatitis is the most common cause.

Most common surgical cause of obstructive jaundice is: Common bile duct (CBD) stones.

Blood supply to liver:

- 60% supplied by portal vein
- 40% supplied by hepatic artery.

- Characterized by **chronic, severe, unconjugated hyperbilirubinemia** → produce severe jaundice, icterus and **death** secondary to kernicterus **within 18 months** of birth.
- **Bile** does contain conjugated bilirubin → hence it is **colorless**.
- Treatment with **phenobarbital** (which induces microsomal enzymes including UGT), has **no effect**.
- **Liver is morphologically normal** by light and electron microscopy. Invariably fatal.

Crigler–Najjar Syndrome Type II

- Less severe, nonfatal disorder.
- Partial deficiency of UGT1A1 enzyme.
- Mode of inheritance—autosomal dominant with variable penetrance.
- **Phenobarbital treatment can improve** bilirubin glucuronidation by inducing hypertrophy of the hepatocellular endoplasmic reticulum.

Gilbert Syndrome

- Relatively **common, harmless**, inherited disorder with no clinical consequences.
- Caused **mutations in the UGT1 gene** → leads to inadequate synthesis of the UGT1A1 enzyme (activity is about 30% of normal), a less severe reduction than in Crigler–Najjar syndromes.
- **Autosomal recessive** mode of inheritance.
- Usually asymptomatic.
- **Mild, chronic unconjugated fluctuating hyperbilirubinemia** not associated with functional derangements.

Dubin–Johnson Syndrome

Q. Write short note on Dubin–Johnson syndrome.

Etiology

Dubin–Johnson syndrome: Liver is darkly pigmented due to melanin-like granules of epinephrine metabolites.

- Benign autosomal recessive disorder.
- Due to the **complete absence of multidrug resistance protein 2 (MRP2)** → defect in hepatocellular excretion

TABLE 19.2: Hereditary hyperbilirubinemias

Hereditary disorder	Mode of inheritance	Defects in bilirubin metabolism	Characteristics
Unconjugated hyperbilirubinemia			
Crigler–Najjar syndrome type I	Autosomal recessive	Absent UGT1A1 activity	Fatal
Crigler–Najjar syndrome type II	Autosomal dominant with variable penetrance	Decreased UGT1A1 activity	Usually mild
Gilbert syndrome	Autosomal recessive	Decreased UGT1A1 activity	Harmless
Conjugated hyperbilirubinemia			
Dubin–Johnson syndrome	Autosomal recessive	Impaired biliary excretion of bilirubin glucuronides	Liver with pigmented granules in cytoplasm, harmless
Rotor syndrome	Autosomal recessive	? Decreased hepatic uptake ? Decreased biliary excretion	Harmless

Abbreviation: UGT, uridine diphosphate–glucuronyl transferase.

Presence of urobilinogen in urine rules out obstructive jaundice.

Multidrug resistance protein 2 (MRP2): A canalicular protein that mediate the transport of conjugated bilirubin and related organic anions across membranes into bile.

of bilirubin glucuronides across the biliary canalicular membrane.

of **hepatocytes** that produces necrosis and inflammation of the liver.

MORPHOLOGY

- Liver is **darkly pigmented** because of **coarse melanin-like pigmented granules** within the enlarged lysosomes present in the cytoplasm of hepatocyte.
- **Pigment composed of polymers of epinephrine metabolites.**

Clinical Features

- Chronic or recurrent conjugated hyperbilirubinemia.
- Most patients are asymptomatic and have a normal life expectancy.

Rotor Syndrome

- Rare form of asymptomatic **conjugated hyperbilirubinemia**.
- Due to many defects, such as hepatocellular uptake, intracellular binding and excretion of bilirubin pigments.
- Inherited as an **autosomal recessive** trait.
- **Liver is morphologically normal.**

VIRAL HEPATITIS

Viral hepatitis: Infection of hepatocytes by viruses.

Definition: Viral hepatitis may be defined as viral **infection**

Cause

Hepatotropic viruses: A, B, C, D, and E. All except HBV are RNA viruses.

Most cases of hepatitis are caused by a group of **five separate, unrelated** viruses that have a particular affinity for the liver known as **hepatotropic virus** (hepatitis viruses A, B, C, D, and E). **All except HBV are RNA viruses.**

- Hepatitis **A and E** cause **infectious** hepatitis and transmitted mainly by the fecal-oral route or ingestion of contaminated water.
- Hepatitis **B, C, and D** cause **serum hepatitis**. They are transmitted mainly by parenteral routes and less commonly by intimate or sexual exposure. They can produce chronic hepatitis, which may progress to cirrhosis and hepatocellular carcinoma.
- **In few cases** of an acute viral hepatitis-like syndrome, the **known hepatitis virus cannot be identified** as etiological agent. They are termed acute non-A, non-B, non-C, non-D, non-E (non-A-E) hepatitis or acute hepatitis of unknown cause.

HAV and HEV: Causes acute hepatitis and never causes chronic hepatitis.

HBV, HCV and HDV: Can cause chronic hepatitis.

Hepatitis A Virus

HAV: Most common viral cause of jaundice.

- **Hepatitis A virus (HAV):** It is a **nonenveloped, 27 nm, RNA virus**. The virus replicates mainly in the liver. It has outer capsid protein (HAVAg).
- **Source of infection:** Only source of infection is **acutely infected person**.
 - Virus is excreted in bile → excreted in stool/feces of infected persons for about 2 weeks before the onset of symptoms and then for a further 2 weeks or so.
 - Disease is **maximally infectious just before the onset of jaundice**.

Mode of Transmission

- **Fecal-oral route** by ingestion of contaminated water and foods. HAV viremia is transient. Hence, blood-borne transmission does not occur.

Incubation period: 3–6 weeks (mean ~ 4 weeks).

Outcome of HAV Infection

- HAV cause a mild, **benign, self-limited acute hepatitis**. HAV **does not produce chronic hepatitis or a carrier state** and fulminant hepatitis develops only rarely.

Laboratory and Serological Findings for HAV Hepatitis

Prodromal Stage

- Serum bilirubin is usually normal.
- **Bilirubinuria** and increased urinary excretion of urobilinogen.
- **Raised serum AST or ALT** precedes the jaundice.

Icteric Stage

- Serum **bilirubin is raised**.
- Serum **AST raised** and maximum levels are reached within 1–2 days after the appearance of jaundice, and may rise above 500 IU/L.
- Serum **ALP is usually less than 300 IU/L**.

Diagnosis (Fig. 19.2)

1. **Demonstration of virus in feces:** The virus can be demonstrated during late incubation period and the preicteric phase by electron microscopy.
2. **Detection of antibody:**
 - **IgM anti-HAV (IgM antibody against HAV):** It appears in blood at the onset of symptoms and is a **reliable marker of acute infection**. It reaches peak levels in 2–3 weeks and disappears after 3–4 months.

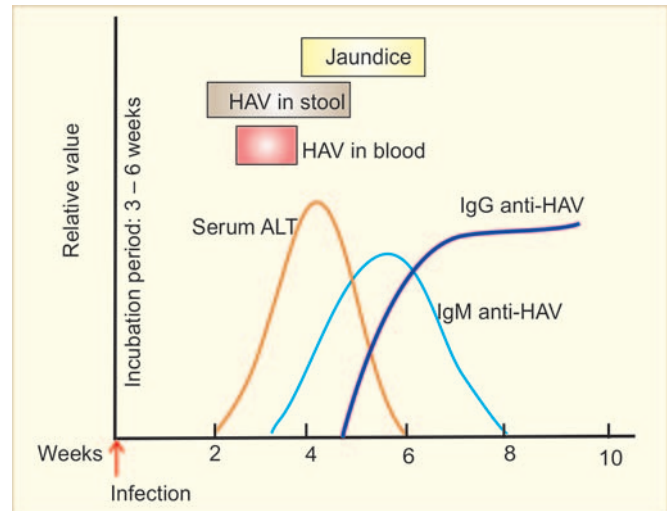


Fig. 19.2: Laboratory and serologic course markers in acute hepatitis A
Abbreviations: ALT, alanine aminotransferase; HAV, hepatitis A virus

- **IgG anti-HAV:** IgM anti-HAV is followed by the appearance of IgG anti-HAV. It persists for years and **provides a lifelong immunity** against reinfection by HAV.

HAV:

- IgM anti-HAV indicates infection
- IgG anti-HAV indicates recovery/vaccination.

Prophylaxis

- Improved sanitary practices and **prevention of fecal contamination of food and water**. HAV vaccine is effective in preventing infection.

Hepatitis B Virus (HBV)

Structure of HBV

- Hepatitis B virus (HBV): It is a **hepatotropic DNA virus** belonging to the family Hepadnaviridae.
- **HBV virion:** It is spherical and **double-layered**.
- **Dane particle:** It is the complete viral particle/virion.

Genome of HBV

It consists of partially double-stranded circular DNA and has four genes (Fig. 19.3).

- **HBsAg (S gene):** HBsAg, hepatitis B surface antigen is a product of S gene, which is **secreted into the blood in large amounts**. HBsAg is **immunogenic**.

HBsAg: Also named Australia antigen, because of its first detection in Australian aborigine.

- **HBcAg (C gene):** The C gene produces two antigenically different products:

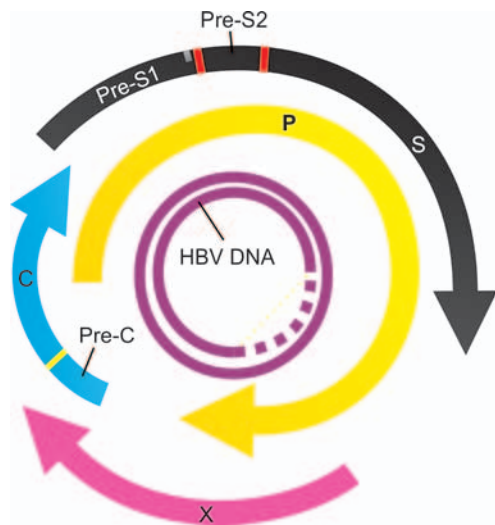


Fig. 19.3: Diagrammatic representation of various genes and respective encoded proteins in HBV. HBV-DNA encodes four proteins namely 1) DNA polymerase required for viral replication (P), 2) surface protein (S), 3) core protein (C) and 4) X protein

- **Hepatitis B core antigen (HBcAg):** It remains intracellular within the hepatocytes and do not circulate in the serum. Hence, not detectable in the serum of patients.
- **Hepatitis B e antigen (HBeAg):** It is secreted into serum and is a surrogate (substitute) marker for high levels of viral replication. It is essential for the establishment of persistent infection.

HBsAg: First viral antigen to appear and last one to disappear with recovery.

- **HBV polymerase (P gene):** A polymerase (Pol) is a product of P gene and DNA polymerase enzyme is needed for virus replication.
- **HBxAg (X gene):** HBx protein is necessary for virus infectivity and has been implicated in the pathogenesis of liver cancer in HBV infection.

Source of infection: Human suffering from hepatitis or carrier is the only source of infection.

HBV is 100 times as infectious as human immunodeficiency virus (HIV) and 10 times as infectious as hepatitis C virus (HCV).

Mode of Transmission

- **Vertical/congenital transmission:** From mother [who is carrier for HBV (90% HbeAg+, 30% HbeAg-ve)] to child may occur in utero, during parturition or soon after birth.
- **Horizontal transmission:** It is the dominant mode of transmission.

– Parenteral:

- ♦ By percutaneous and mucous membrane: Exposure to infectious body fluids, through minor cuts/abrasions in the skin or mucous membranes. HBV can survive for long periods on household articles, e.g. toys, toothbrushes and may transmit the infection.
- ♦ Intravenous route: Through transfusion of un-screened infected blood or blood products. This mode of spread is rare now, because of routine screening of all blood donors for HBV and HBC. Other modes include intravenous drug abuse with sharing of needles and syringes, tattooists or acupuncturists.

- **Close personal contact:** Unprotected heterosexual or homosexual intercourse. The virus can be found in semen and saliva.

Incubation period: It ranges from 4 to 26 weeks.

Four Bs of mode of transmission of HBV:

- Blood
- Birth
- Blood bank
- Body fluids.

HBV is not transmitted by breastfeeding.

Sequelae/Outcome of HBV Infection (Fig. 19.4)

Q. Write short note on sequelae of hepatitis B virus infection.

1. **Acute hepatitis** with recovery and clearance of the virus.
2. **Chronic hepatitis.**
3. Progressive chronic disease ending in **cirrhosis**.
4. **Fulminant hepatitis** with massive liver necrosis.
5. Asymptomatic **carrier** state.
6. Hepatocellular carcinoma.

Sequence of Serological Markers for HBV Hepatitis

Q. Discuss the laboratory diagnosis (serological markers) of hepatitis B virus infection.

The natural course of the disease can be followed by serum markers (Fig. 19.5).

HBsAg

HBsAg: Persistence after 6 months indicates chronic HBV.

- It is the **first virologic marker**, which appears in serum before the onset of symptoms. It peaks during the disease and becomes undetectable within 3–6 months.

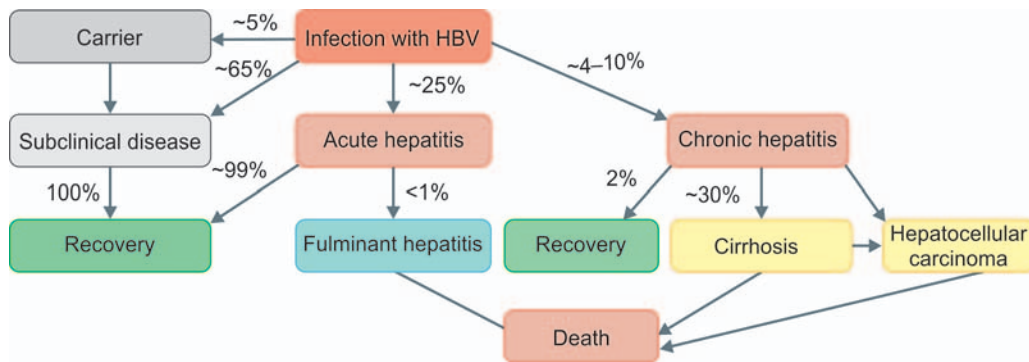
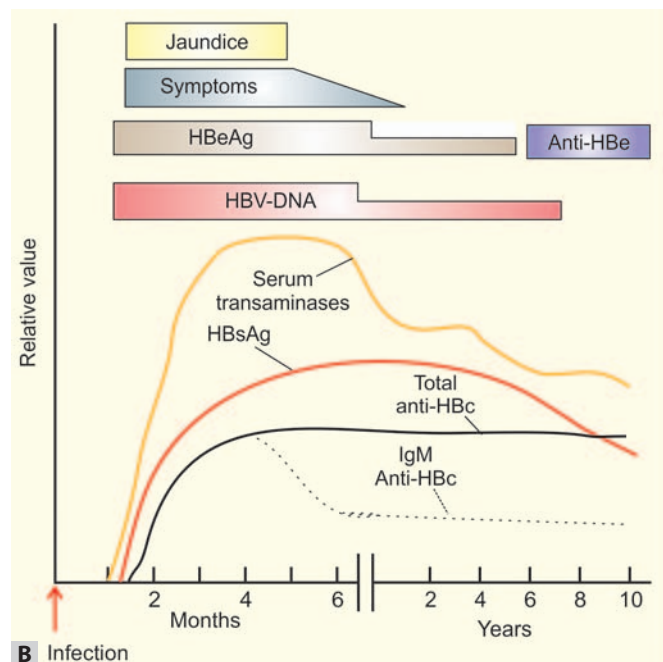
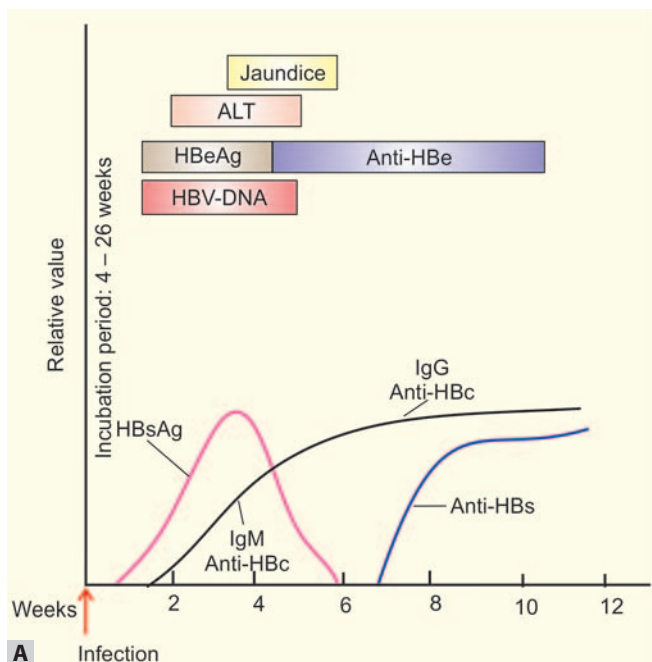


Fig. 19.4: Potential outcomes of hepatitis B infection



Figs 19.5A and B: Sequence of serologic markers: (A) Acute hepatitis with resolution; (B) Chronic hepatitis caused by HBV

- **Significance:**
 - Present in the serum in both acute and chronic hepatitis B; indicates an infectious state. Loss of HBsAg plus the development of anti-HBs denotes recovery.

Carrier state: Presence of HBsAg in the serum for 6 months or more after the initial detection.

Anti-HBs

Anti-HBs: Protective antibody; develops after recovery/immunization.

- It is antibody to HBsAg and detectable in serum after the disappearance of HBsAg.

- **Significance:** Anti-HBs is a **protective antibody** and present in the serum in the recovery phase and in immunity (i.e. vaccination) and **may persist for life providing protection**. This is the basis for current vaccination using noninfectious HBsAg.

HBeAg, HBV-DNA, and DNA Polymerase

HBeAg and HBV-DNA: Infective particles of HBV.

- They appear in serum soon after HBsAg.
 - Significance:** Usually not helpful in the diagnosis of hepatitis B, but may be **valuable in assessing prognosis**. They indicate active viral replication.

TABLE 19.3: Summary of serological findings in HBV

Antigens		Antibodies			Interpretation
HBsAg	HBeAg	Anti-HBc	Anti-HBs	Anti-HBe	
+	+	IgM	-	-	Acute hepatitis B, highly infectious
+	+	IgG	-	-	Chronic infection or carrier state, high infectivity
+	-	IgG	-	+/-	Chronic infection or carrier state, low infectivity
-	-	-	+	-	Immunity following HBV vaccine

Testing for HBeAg, anti-HBe, HBV DNA, and anti-HBs does not help in the diagnosis of hepatitis B but may be valuable in assessing prognosis.

- **HBeAg or HBV- DNA**
 - ◆ Their persistence 6 weeks after the onset of symptoms indicates infectivity and probably develop chronic hepatitis B.
 - ◆ Their absence is a favorable serologic finding and if associated with appearance of anti-HBe antibodies indicates low infectivity.
- **Anti-HBe** is present in the serum in the recovery phase.

HBcAg: Never appears in the blood.

Anti-HBc

Marker during window period of HBV: IgM anti-HBc.

- **HBcAg is not found in the serum.** But its antibody, IgM anti-HBc appears in serum a week or two after the appearance of HBsAg. After about 6 months, the IgM anti-HBc antibody is replaced by IgG anti-HBc.
- **Significance:**
 - IgM anti-HBc is the **earliest antibody marker** seen in the serum, long before anti-HBe or anti-HBs.
 - **IgM anti-HBc indicates recent infection** (first 6 months).
 - **IgG anti-HBc indicates remote infection** (beyond 6 months). IgG anti-HBc remains lifelong in the serum and its presence **indicates previous infection with HBV** even when all the other viral markers are not detectable.

Most useful indicator of prior infection with HBV is anti-HBc Ag.

IgG anti-HBc: Present after 6 months of infection.

Serological findings in HBV are summarized in Table 19.3.

Prevention

Hepatitis B can be prevented by **vaccination** and by the **screening of donor blood, organs, and tissues**. The vaccine

is **purified HbsAg** and induces a protective anti-HBs antibody response.

Hepatitis C Virus

- HCV is a **small, enveloped, single-stranded RNA virus**. It is a member of the Flaviviridae family.
 - A characteristic feature is **emergence of an endogenous, newly mutated strain**. Because of this **genomic instability and antigenic variability**, producing an effective HCV vaccine is difficult.

Mode of spread: It mainly spreads by the **parenteral route** as a blood-borne infection. It may also spread by **sexual contact**.

Incubation period: 2–26 weeks (mean 6–12 weeks).

Sequelae/Outcome of HCV Infection

Q. Sequelae of hepatitis C virus infection.

1. **Acute hepatitis.**
2. **Chronic hepatitis:** It occurs in the majority of individuals infected by HCV. It can be prevented by screening procedures.
3. **Cirrhosis:** It develops over 5–20 years in 20–30% of patients.
4. Fulminant hepatic failure is rare.

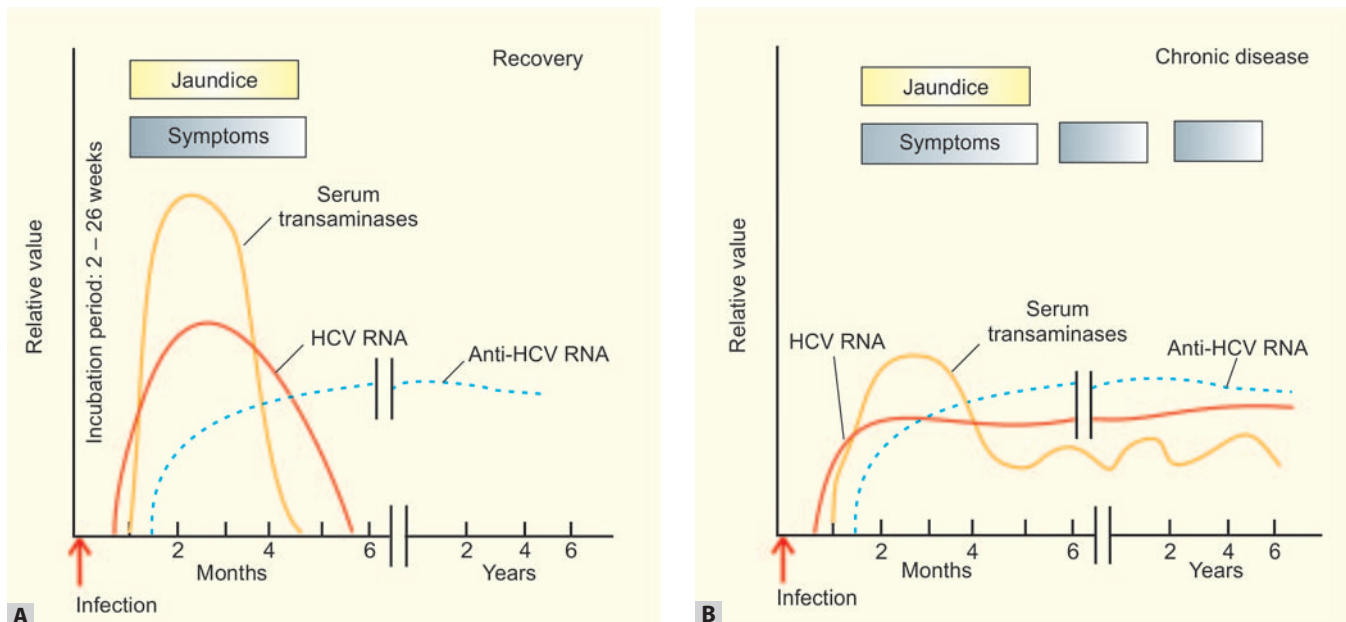
Serum markers HCV hepatitis (Figs 19.6A and B): The elevated titers of **IgG anti-HCV** after an active infection **do not confer effective immunity**.

Two Cs of HCV:

- Chronic hepatitis—more often
- Cirrhosis.

HBV and HCV infections:

Increased risk of HCC even in the absence of cirrhosis.



Figs 19.6A and B: (A) Sequence of serologic markers for acute hepatitis caused by HCV; (B) Sequence of serologic markers for chronic hepatitis caused by HCV

Hepatitis D Virus

Three Ds of HDV:

- Delta agent
- Defective virus
- Depends on HBV.

Hepatitis D virus (HDV) is a **defective RNA virus**, which requires HBV for its replication and expression. Because HDV is dependent on HBV, the duration of HDV infection is determined by the duration of HBV infection.

Patterns of HDV Hepatitis

HDV causes **Delta hepatitis** with two clinical patterns.

- **Acute coinfection:** It develops when an individual is **exposed simultaneously** to serum containing **both HDV and HBV**. The HBV infection first becomes established and the HBsAg is necessary for development of complete HDV virions.
- **Superinfection:** It occurs when an **individual already infected with HBV** is exposed to a **new dose HDV**.

Mode of spread: Parenteral route and sexual contact.

Outcome of HDV Infection

HCV and HDV: Does not produce protective antibody.

- **Coinfection of HBV and HDV**
 - **Acute hepatitis B + D:** It is usually transient and self-limited and clinically similar to acute hepatitis B.
 - **Chronic hepatitis:** It is similar to acute hepatitis B.

- **Superinfection with HDV in a chronic HBsAg carrier**
 - **Acute hepatitis:** It may be severe in a HBV carrier, or chronic hepatitis B.
 - **Chronic hepatitis.**
 - **Cirrhosis and hepatocellular cancer (HCC).**

Serological Markers of HDV

HDV: Defective virus; causes hepatitis with HBV co-infection or superinfection.

- **HDV RNA:** It is detectable in the blood and liver **before and in the early days of acute disease**.
- **Anti-HDV:** IgM anti-HDV-most reliable indicator of recent HDV exposure.

Chronic viral hepatitis: Produced by HBV, HCV and HDV.

Hepatitis E Virus

- HEV is an unenveloped, RNA virus in the **Hepevirus** genus. Viral particles are 32-34 nm in diameter.
- HEV infection is responsible for more than 30-60% of cases of sporadic acute hepatitis in India.
- Hepatitis E occurs primarily in young to middle-aged adults.

Source of infection: HEV is a **zoonotic disease with animal reservoirs**, such as monkeys, cats, pigs, and dogs. Virions are shed in stool during the acute illness.

Mode of transmission: It is an **enterically transmitted, water-borne** infection.

Incubation period: ~6 weeks.

Outcome of HEV Infection

HEV: High mortality rate (about 20%) among pregnant women.

It causes **self-limiting acute hepatitis**. It does not cause chronic liver disease. But it has a **high mortality rate (about 20%) among pregnant women**.

Diagnosis of HEV

- Before the onset of clinical illness, HEV RNA and HEV virions can be detected in stool and serum.
- After the onset of clinical illness, serum aminotransferases rise and elevated IgM anti-HEV titers also occur simultaneously. After recovery, the IgM is replaced with a persistent IgG anti-HEV titer.

Three Es of HEV

- Endemic in equatorial regions
- Epidemic frequent
- Expectant mother have high mortality.

Salient features of hepatitis viruses are presented in Table 19.4.

HAV and HEV: Cause only acute hepatitis and never chronic hepatitis.

Clinical Features

1. Asymptomatic acute infection with recovery (serologic evidence only).
2. Acute hepatitis (anicteric or icteric).
3. Fulminant hepatitis with massive to submassive hepatic necrosis.

4. Chronic hepatitis (without or with progression to cirrhosis).
5. Chronic carrier state.

Phases of acute hepatitis: Preicteric, icteric and convalescence.

Acute Asymptomatic Acute Infection with Recovery

It is usually incidentally identified because of mild elevation of serum transaminases or presence of antiviral antibodies.

Acute Hepatitis

It can be caused by any one of four hepatotropic viruses. This can be divided into four phases:

- i. **Incubation period** (varies depending on the type of virus).
- ii. **Symptomatic preicteric phase:** It presents with non-specific symptoms, such as malaise, nausea, poor appetite, and vague abdominal pain.
- iii. **Symptomatic icteric phase:** Jaundice and yellow sclera, dark colored urine (conjugated hyperbilirubinemia), and pruritis (bile salt retention).
- iv. **Convalescence**

Investigations

Acute hepatitis: Aminotransferase levels peak before the appearance of jaundice.

Acute hepatitis: Serum ALT is the last enzyme to return to normal.

TABLE 19.4: Summary of features of hepatitis viruses

Type	Type of virus	Route of transmission	Mean incubation period	Frequency of chronic liver disease	Diagnosis
A	ssRNA	Fecal-oral	2–4 weeks	Never	IgM anti-HAV
B	Partially dsDNA	Parenteral, sexual contact, perinatal	1–4 months	10%	HBsAg or antibody to HBcAg
C	ssRNA	Parenteral	7–8 weeks	~80%	Anti-HCV, HCV RNA
D	Circular defective ssRNA	Parenteral	Same as HBV	5% (coinfection); ≤70% (superinfection)	Anti-HDV, HDV RNA; Coinfection—IgM anti-HBc and anti-HDV; Superinfection—IgG anti-HBc and anti-HDV
E	ssRNA	Fecal-oral	4–5 weeks	Never	IgM/IgG anti-HEV

Abbreviations: ss, single stranded; ds, double-stranded DNA; HBcAg, hepatitis B core antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis D virus; HEV, hepatitis E virus.

Less common causes of viral hepatitis:

1. Cytomegalovirus
2. Epstein–Barr virus.

Rare causes of viral hepatitis:

1. Herpes simplex
2. Yellow fever.

- **Biochemical:** Raised serum bilirubin and aminotransferase levels.
- **Serology:** Hepatitis viral genome in the liver and serum followed by antibodies to viral antigens.
- **Microscopy:** Varying degrees of necrosis of hepatocyte and inflammation (refer page 536).
 - Chronic hepatitis: It may be without or with progression to cirrhosis (refer page 537)
 - Chronic carrier state (discussed below)

Fulminant Hepatitis

- Fulminant hepatitis with massive to submassive hepatic necrosis (refer page 538).

Carrier State in HBV

Definition: A “carrier” is an individual who harbors and can transmit an organism (HBV), but does not manifest symptoms.

Factors Determining Carrier State

- **Age at infection:** If infection occurs in children perinatally → high rate of developing carrier state. The carrier rate is lowest when adults are infected.
- **Impaired immunity** → carrier state.

Types of Carriers

Types of carriers:

- Healthy carriers
- Active carriers.

Carrier state constitutes reservoirs for infection.

- **Inactive carriers (healthy carriers):** They carry one of the viruses but have no liver disease. They have **normal or only mildly raised serum aminotransferase** values **without HBeAg**, but **show anti-HBe**. Majority do not progress to liver disease.
- **Active carriers:** They harbor the viruses and have non-progressive liver damage. They are usually asymptomatic, but show intermittent or persistent **elevation of serum aminotransferase levels**.

Active/infective carrier of HBV:

- HBsAg+
- HBeAg+
- HBV-DNA+
- IgG anti-HBc+.

Morphology

The liver cells of carriers show **ground glass appearance** due to HBsAg in the cytoplasm (Fig. 19.7). These cells **stain orange** with Orcein stain.

Morphology of Acute and Chronic Hepatitis

Q. Morphology of liver in viral hepatitis.

- **Acute versus chronic hepatitis:** Differentiation is by duration and microscopic pattern of cell injury. Hepatitis may be caused by viruses as well as other agents (e.g. drugs, toxins, autoimmune).

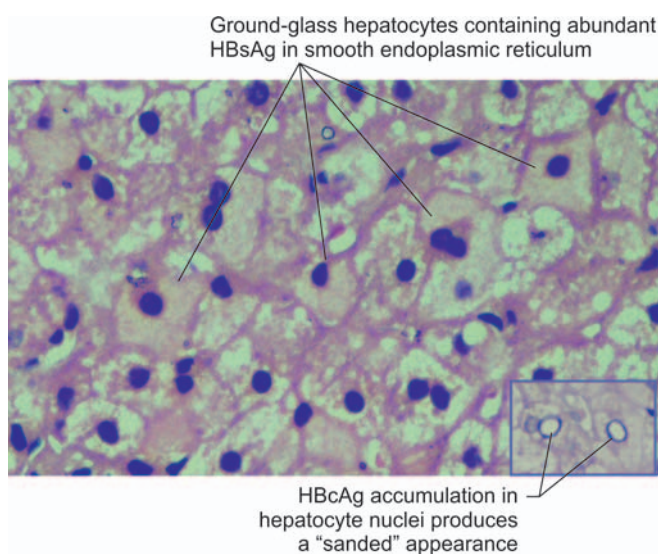


Fig. 19.7: Chronic HBV carrier showing few liver cells with diffuse granular cytoplasm giving rise to ground-glass hepatocytes due to abundant HBsAg in the smooth endoplasmic reticulum. Inset shows hepatocyte nuclei with sanded appearance due to accumulation of HBcAg

HBsAg: Responsible for ground-glass hepatocytes.

Ground-glass hepatocytes: HBV-infected liver cells having finely granular cytoplasm packed with spheres and tubules of HBsAg.

HBcAg gives sanded nuclei appearance.

Healthy/inactive carrier of HBV:

- HBsAg+
- IgG anti-HBc+.

- **General morphological features:** Majority of microscopic changes caused by hepatotropic viruses (A, B, C, D and E) are generally similar.

Acute Hepatitis

MORPHOLOGY

Gross

- Normal or may be swollen in acute hepatitis
- Involvement may be **diffuse or patchy**
- Cut section muddy-red, mushy with yellow or green discoloration due to jaundice

Microscopy (Fig. 19.8)

Inflammation is scattered throughout the hepatic lobule and is termed as “spotty necrosis” or lobular hepatitis. Inflammatory cells: Both in acute and chronic viral hepatitis are mainly T cells.

1. Hepatocyte injury:

- **Ballooning degeneration:** It is seen with mild injury. It is characterized by **swelling of hepatocytes**, empty and pale-stained cytoplasm, with clumping of cytoplasm around the nucleus.
- **Hepatocyte necrosis:**
 - ♦ **Dropout necrosis:** Rupture of the cell membrane of ballooned hepatocytes → leads to cell death and focal loss of hepatocytes → necrotic cell **dropout** → collapse of sinusoidal collagen reticulin framework → **aggregates of macrophage** around necrotic hepatocyte.
 - ♦ **Acidophilic or apoptotic or Councilman body:** It is caused by anti-viral cytotoxic (effector) T cells. Apoptotic hepatocytes shrink → become **intensely eosinophilic** and have a densely staining **pyknotic or fragmented nuclei**. They may be surrounded by effector T cells. The remnants of apoptotic hepatocytes may be extruded into the sinusoids → appear as **acidophilic or Councilman bodies**.

- ♦ **Bridging necrosis** (Fig. 19.9): It is a confluent (uniting band/zone) necrosis of hepatocytes observed in severe acute hepatitis. This **band/zone of necrosis** may extend from **portal tract to portal tract, central vein to central vein, or portal-to-central regions of adjacent lobules**.

Councilman bodies: Acute viral hepatitis.

- Inflammation:** It involves **all areas** of the lobule and is a **characteristic and prominent feature** of acute hepatitis.
 - **Mononuclear inflammatory cells:** The inflammatory cells in acute hepatitis mainly consists of **lymphocytes and macrophages**.
 - **Lobular hepatitis:** It is **inflammation involving liver parenchyma away from portal tract**. Unlike chronic hepatitis, the inflammatory infiltrate is usually not concentrated in portal tracts but is seen throughout the lobule.
 - **Interface hepatitis:** It can **occur in acute and chronic hepatitis** (refer page 537).
- Kupffer cells:** They show **hypertrophy and hyperplasia** and contain lipofuscin pigment as a result of phagocytosis of hepatocellular debris.
- Lobular disarray:** It is due to combination of necrosis of hepatocytes, accompanying regeneration and mononuclear inflammatory infiltrate → disruption of the normal orderly architecture of the liver cell plates. Rarely **cholestasis** may be found, characterized by the bile plugs in canaliculi and brown pigmentation of hepatocytes.

Microscopy of acute hepatitis:

- Hepatocyte injury
- Inflammation
- Sinusoidal cell (Kupffer cells) reactive changes
- Lobular disarray.

Chronic hepatitis C: Also shows

- Steatosis (fatty change)
- Lymphoid aggregates in the portal tract
- Bile duct damage.

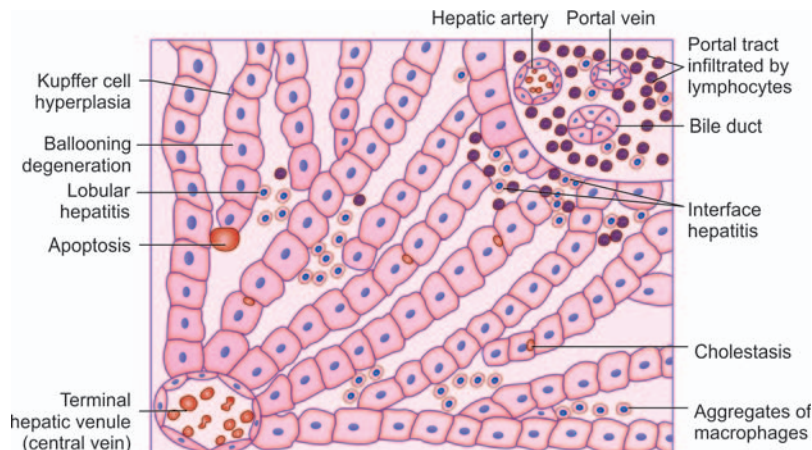


Fig. 19.8: Microscopic features of acute hepatitis

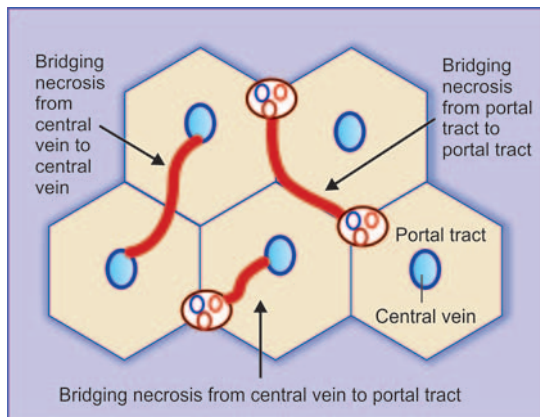


Fig. 19.9: Patterns of bridging necrosis in severe hepatitis

Bridging necrosis : Band of necrosis from

- Portal tract to portal tract
- Central vein to portal tract
- Portal tract to central vein of adjacent lobule.

CHRONIC HEPATITIS

Chronic hepatitis: Symptomatic, biochemical, or serologic evidence of hepatic disease for more than 6 months.

Definition: Chronic hepatitis is defined as **symptomatic, biochemical, or serologic evidence of hepatic disease for more than 6 months**. Microscopically, there should be inflammation and necrosis in the liver.

Causes

Hepatitis may be caused by viruses as well as other etiological agents (Table 19.5). The viruses include:

- **HCV:** It is the most common cause of chronic viral hepatitis and mostly asymptomatic.
- **HBV:** Chances of chronic hepatitis is high if the **infection occurs at a younger age**. **Maternal-to-infant transmission** is a major risk factor.
- **HDV+HBV:** Either superinfection or co-infection → chronic hepatitis.

TABLE 19.5: Major causes of chronic hepatitis

Virus	Other causes
Chronic hepatitis B	Autoimmune hepatitis
Chronic hepatitis C	Drug-induced chronic hepatitis
Chronic hepatitis D	Wilson's disease
	Cryptogenic hepatitis (non-A–E hepatitis)

MORPHOLOGY

Q. Write short note on chronic hepatitis.

Gross

- **Early stage:** Liver may appear **normal**.
- **Later stage:** Liver feels **firm** because of increased fibrosis → may progress to macronodular cirrhosis.

Microscopy (Fig. 19.10)

Range from mild to severe hepatitis. Regardless of etiology, it is characterized by a combination of 1) portal inflammation, 2) interface hepatitis, 3) parenchymal inflammation and necrosis, and 4) fibrosis.

1. **Portal inflammation:** In **mild hepatitis**, inflammation is **limited to portal tracts** and predominantly consists of **lymphocytes, macrophages, and occasional plasma cells**.
2. **Interface hepatitis (piecemeal necrosis/periportal necrosis):** It is an **important feature** characterized by **spillover of inflammatory cells** (lymphocytes and plasma cells) **from portal tract into the adjacent parenchyma** at the limiting plate → associated with degenerating and apoptosis of periportal hepatocytes.

Interface hepatitis: Spillover of inflammatory cells from portal tract into the adjacent parenchyma at the limiting plate.

3. **Parenchymal inflammation and necrosis:** It is variable in severity but usually spotty. Mononuclear inflammatory cells surround the damaged hepatocytes. **Bridging necrosis** between portal tracts and portal tracts-to-terminal hepatic veins **may be seen**.
4. **Fibrosis:** It is the **hallmark of chronic liver damage**. Continued inflammation and associated necrosis → leads to progressive fibrosis at the limiting plate and enlargement of the portal tract.
 - a. **Initially:** Fibrosis is seen only in the portal tracts.
 - b. **Later:** Periportal septal fibrosis occurs. This is followed by bridging /linking of fibrous septa (bridging fibrosis) between adjacent fibrotic portal tracts (i.e. portal to portal) or portal-central.

Scoring systems: Used to assess the severity and progression of liver damage due to HBV and HCV infection. Key elements in these systems are as follows: Inflammation and hepatocyte destruction (grade), and the severity of fibrosis (stage).

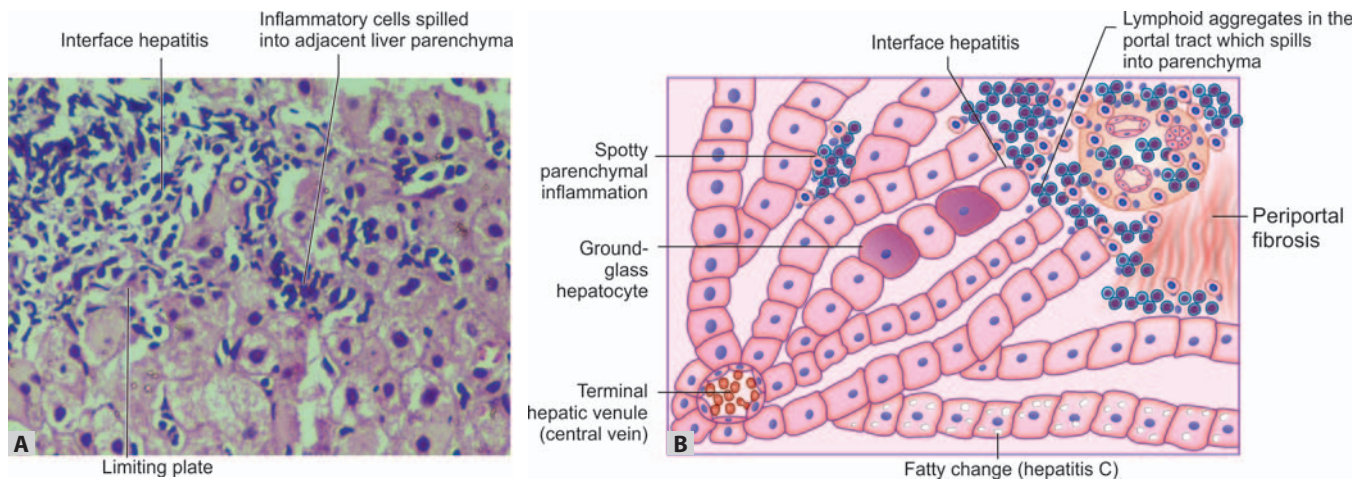
Clinical Features

Highly variable.

- **Fatigue:** It is the most common symptom.
- **Other symptoms:** Malaise, loss of appetite, and bouts of mild jaundice.

Physical Findings

These are few, such as spider angiomas, palmar erythema, mild hepatomegaly, hepatic tenderness, and mild splenomegaly.



Figs 19.10A and B: Microscopic features of chronic hepatitis: (A) Photomicrograph; (B) Diagrammatic

Laboratory Findings

- Raised serum transaminase, prolonged prothrombin time, hyperbilirubinemia, and mild elevation of alkaline phosphatase level. Urine shows increased bilirubin and urobilinogen.

Consequence

- Continued loss of hepatocytes and fibrosis → results in **cirrhosis**. Cirrhosis is characterized by irregularly sized nodules separated by broad fibrous scars and is referred to as **post-necrotic cirrhosis**. It is usually **macronodular or mixed micro- and macronodular** type. The term post-necrotic is not specific and is applied to any cirrhosis in which the liver shows large, irregular-sized nodules with broad scars.

Chronic infection by HBV and HCV: Increased risk for hepatocellular carcinoma.

Causes of post-necrotic cirrhosis: Viral hepatitis, autoimmune hepatitis, hepatotoxins (carbon tetrachloride, mushroom poisoning), drugs (acetaminophen, α -methyl dopa), and rarely alcohol.

Cryptogenic cirrhosis: Cause not known.

Fulminant Hepatic Failure

Definition: Hepatic insufficiency progresses **within 2–3 weeks** from **onset of symptoms** to hepatic encephalopathy, in patients who do not have chronic liver disease.

Fulminant hepatic failure: Hepatotrophic virus is the most common cause.

Causes

- **Viral hepatitis:** HBV and HAV. Occasionally, HCV and others.
- **Noninfectious causes:** Acetaminophen toxicity.
- Unknown.

Pathogenesis

- It varies depending on etiology. HBV-induced fulminant hepatitis shows massive apoptosis and factors, which include advanced age and female sex.
- When the destruction is massive, regeneration is disorderly and result in nodular masses of liver cells. Fibrous scarring may lead to cirrhosis.

MORPHOLOGY

Morphological features are almost similar irrespective of the causative agent. It can be subdivided into submassive and massive necrosis.

Gross

- Distribution of liver destruction varies.
- May involve **the entire liver or only random areas**.
- **Massive necrosis:** Liver may shrink → 500–700 g. It appears **shrunken, red covered by a wrinkled, too-large capsule**. Cut section, the **necrotic areas appear muddy red, mushy** with areas of hemorrhage.

Fulminant hepatic failure:

- Liver shrinks to 500–700 g
- Wrinkled capsule
- Massive necrosis of hepatocytes
- Minimal inflammatory reaction.

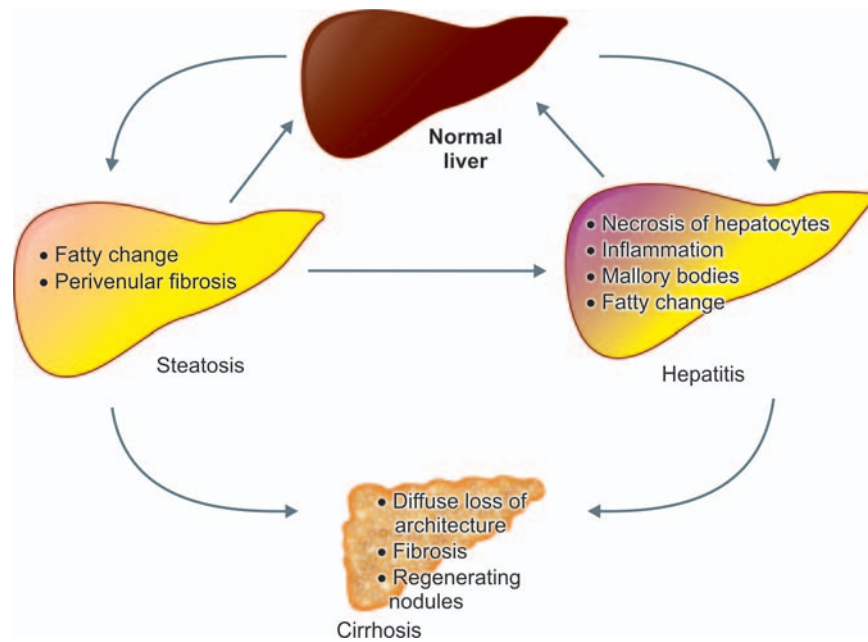


Fig. 19.11: Interrelationships among steatosis, hepatitis, and cirrhosis in alcoholic liver disease

Microscopy

- **Massive necrosis of hepatocytes** in contiguous lobules and the reticulin framework is collapsed in these regions.
- **Minimal inflammatory reaction.**
- If patient survives for several days, there may be inflammatory cells that phagocytose the necrotic cells.
- If the parenchymal framework is preserved, regeneration can completely restore the liver architecture.

Prognosis: The mortality is ~80% without liver transplantation, and ~35% with transplantation.

ALCOHOLIC LIVER DISEASE

Alcoholic liver disease Includes:

1. Hepatic steatosis
2. Alcoholic hepatitis
3. Alcoholic cirrhosis.

- Chronic and excessive alcohol (ethanol) consumption is **one of the major causes** of liver disease.
- Alcoholic liver disease (ALD) constitutes a **spectrum of disorders** directly related to the excessive alcohol use.
- ALD consists of **three major, distinctive, but overlapping lesions** (Fig. 19.11): (1) Hepatic steatosis (fatty liver), (2) alcoholic hepatitis, and (3) alcoholic cirrhosis.

Metabolism of Ethanol (Fig. 19.12)

The liver is the main organ involved in the ethanol metabolism.

Formation of Acetaldehyde

Ethanol is metabolized to acetaldehyde by three enzyme systems present in the liver namely: 1) Alcohol dehydrogenases (ADHs), 2) cytochrome P450 2E1 (CYP2E1), and 3) catalase (least important).

1. **Alcohol dehydrogenase (ADH):** It is present in the cytoplasm of the liver and is the **main enzyme system involved in alcohol metabolism at low concentrations.**
2. **Cytochrome P450 2E1 (CYP2E1):** When blood alcohol levels are high, the **microsomal** (present in microsomes) **ethanol-oxidizing system (MEOS)** participates in its metabolism. The enzyme involved is cytochrome P450 2E1 (CYP2E1). It **can also generate reactive oxygen species.**
3. **Catalase:** It is present in peroxisomes and is of **minor importance.**

Formation of Acetic Acid

- Acetaldehyde, **is converted to acetic acid** by acetaldehyde dehydrogenase (ALDH) in the mitochondria. The acetic

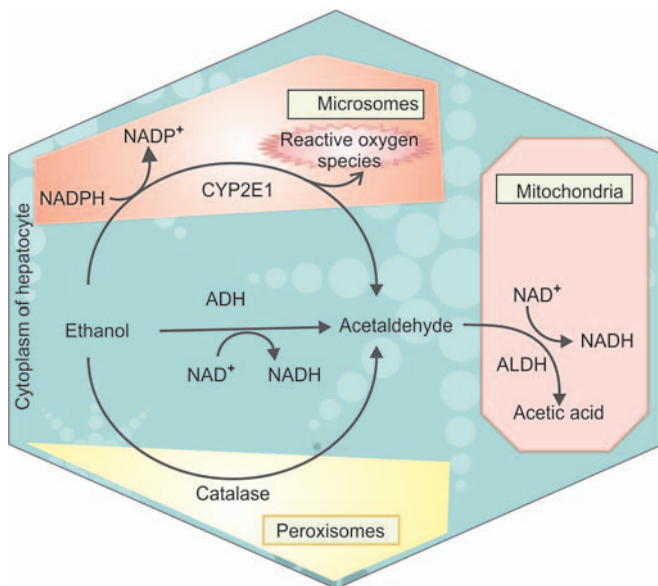


Fig. 19.12: Ethanol metabolism in hepatocyte. Ethanol is converted to acetaldehyde by three different routes: 1) Most important route is in the cytosol by alcohol dehydrogenase (ADH) 2) in the microsomes (by CYP2E1), and 3) in the peroxisomes (by catalase). Acetaldehyde is oxidized by aldehyde dehydrogenase (ALDH) to acetic acid in mitochondria. Oxidation through CYPs in the microsomes may also generate reactive oxygen species NADP^+ = Nicotinamide-adenine dinucleotide phosphate, NADPH =reduced form of nicotinamide-adenine dinucleotide phosphate. NAD^+ =nicotinamide adenine dinucleotide, NADH =reduced form of nicotinamide adenine dinucleotide

acid is then utilized in the mitochondrial respiratory chain.

Etiology of Alcoholic Liver Disease

Q. Discuss the pathogenesis of alcoholic cirrhosis.

Risk Factors

They influence the development and severity of alcoholic liver disease.

Risk of ALD:

- Variable and not everyone who drinks heavily will develop alcoholic liver disease
- Cirrhosis develops after 10–15 years.
- **Gender:** Females are more susceptible to ALD than males. Females develop advanced liver disease with substantially less alcohol intake. Estrogen increases gut permeability to endotoxins (gut-derived) and increased production of pro-inflammatory cytokines and chemokines (from macrophages and Kupffer cells) resulting in injury to liver cells.

- **Ethnicity:** Irrespective of amount of alcohol consumed, ethnic difference is noticed in alcohol-induced liver damage.
- **Genetic factors:** Genetic predisposition is likely and **polymorphisms in detoxifying enzymes** (aldehyde-dehydrogenase-ALDH) have been identified.
- **Associated conditions:** **Iron overload and chronic infections with HCV and HBV can increase the severity of ALD and hasten the progression of alcoholic liver disease to cirrhosis in chronic alcoholics.** Even **moderate alcohol intake increases the risk of cirrhosis and hepatocellular cancer in HCV-infected patients.**

Drinking pattern and liver disease:

- Type of beverage does not affect risk
- Damage is more in continuous rather than binge drinkers.
- **Amount and duration of alcohol intake** (drinking patterns): These are the **most important risk factors**. The time required to develop ALD is **directly related to the amount of alcohol consumed**. Consumption of moderate amounts of alcohol is usually not injurious, but excessive amounts causes damage.
 - Short-term ingestion of about 80 g of alcohol (six beers or 8 ounces of 80-proof liquor) over one to several days → produces mild, reversible hepatic steatosis.
 - Daily intake of 80 g or more of ethanol → increases the risk for severe hepatic injury.
 - Daily consumption of 160 g or more for 10–20 years → severe injury.

Pathogenesis of Alcoholic Liver Disease

Pathogenesis of alcoholic liver injury is not completely known. Alcohol is a **direct hepatotoxic** and **its metabolism in the liver initiates several pathogenic process**. About 10–15% of alcoholics develop cirrhosis (Fig. 19.13).

Mechanisms of Liver Injury by Ethanol

(Fig. 19.13)

Alcoholic hepatitis: Alcohol is converted to acetaldehyde in the liver and directly or indirectly damages the hepatocytes.

Ethanol is metabolized to a highly reactive and potentially toxic compound namely **acetaldehyde** in the liver (Fig. 19.12). Acetaldehyde **plays an etiologic role in alcoholic liver disease**. The oxidation of ethanol produces several toxic agents and damages the metabolic pathways. The most important mechanisms are discussed below:

Oxidative Stress/Reactive Oxygen Species

Acetaldehyde: Highly reactive and potentially toxic compound.

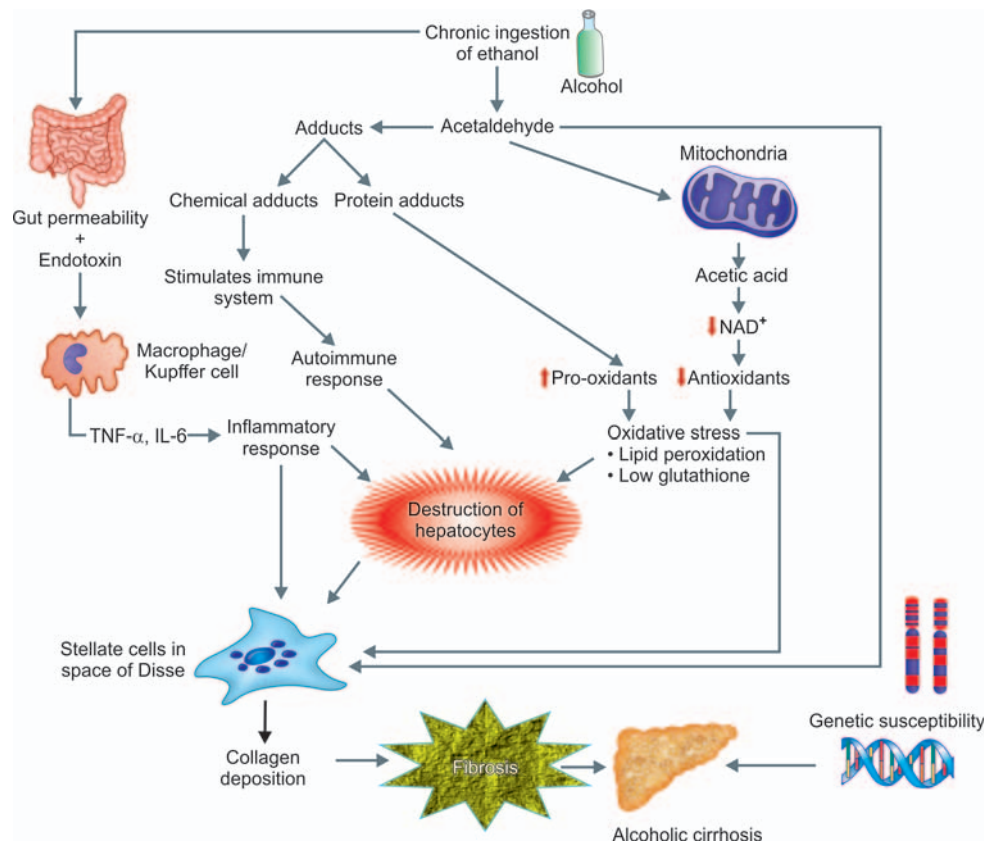


Fig. 19.13: Pathogenesis of alcoholic liver disease

Metabolism of alcohol in the liver by CYP2E1 in the microsomes produces **reactive oxygen species** (such as hydrogen peroxide and superoxide ions), which **cause lipid peroxidation** of cell membranes leading to injury (refer page 15) to hepatocyte.

Immune and Inflammatory Mechanisms by Forming Chemical Adducts

Acetaldehyde forms chemical adducts with cellular proteins in hepatocytes and form neoantigens → which initiate immune response → cause **cell injury similar to autoimmune-like diseases**.

Increased Redox (NADH:NAD⁺) Ratio

- This is due to decreased NAD and may occur in both cytoplasm as well as the mitochondria of hepatocytes.
- **Decreased NAD⁺ in cytoplasm:** Oxidation of ethanol in the cytoplasm of hepatocytes by ADH decreases the amount of nicotinamide adenine dinucleotide (NAD⁺) and increase in NADH (reduced form NAD). Decreased NAD⁺ **inhibits oxidation of fatty acid** and leads to **accumulation of fat** in the liver. It may also cause lactic acidosis.

- **Mitochondrial dysfunction:** The **acetaldehyde** formed from ethanol is **converted to acetic acid in mitochondria**. This reaction also increases, causes NADH/NAD⁺ ratio in the mitochondria and generates reactive oxygen species (superoxide ions). Normally, antioxidant glutathione is transported from the cytoplasm into the mitochondria and can neutralize oxidants. This transport of glutathione is impaired in alcoholic liver disease. Due to **depletion of glutathione**, the generated reactive oxygen species produce **mitochondrial dysfunction**.

Effects of increased redox (NADH:NAD⁺) ratio:

1. Accumulation of fat
2. Mitochondrial dysfunction
3. Lactic acidosis.

Increased Production of Proinflammatory Cytokines

- **Lipopolysaccharide from gram-negative bacteria:** In the intestinal flora, alcohol causes the release of endotoxin (lipopolysaccharide-LPS) from gram-negative bacteria. **LPS enter the portal circulation** → **stimulates production of TNF- α** (tumor necrosis factor α) and other

cytokines (IL-6, and TGF- α) from macrophages and Kupffer cells → produces **injury to liver cells**.

- **Impaired proteasome function:** Normal function of the ubiquitin-proteasome pathway is to remove irregular and damaged proteins. In alcoholic cirrhosis, the **function of proteasome is impaired** → **inefficient degradation of ubiquitin** → **accumulation of large amounts of ubiquitin** in the hepatocytes in the form of **Mallory bodies**. Impaired proteasome function also causes → death of hepatocytes and release cytokines, such as interleukin (IL)-8 and IL-18. IL-8 attracts neutrophils and IL-18 sustains inflammation and causes damage to liver cells.

Direct Toxicity by Forming Protein Adducts

- Acetaldehyde can form adducts with reactive residues on proteins or small molecules (e.g. cysteines) and form toxic molecules which can directly damage the hepatocytes. This is in addition to damage produced by immunological mechanisms mentioned above.

Hypoxic Damage

- The centrilobular area of the hepatic lobule has the lowest oxygen tension and high susceptibility to hypoxia induced damage. **Chronic alcohol consumption** increases oxygen demand by the liver resulting in a hypoxia of the centrilobular region.

Reduced Levels of ADH and ALDH Isozymes

- The **efficiency of alcohol metabolism** in the liver **depends on** the expression levels of **ADH and ALDH isozymes**. Persons having genetic variants with low ALDH activity cannot oxidize acetaldehyde and cannot tolerate alcohol.

Abnormal Metabolism of Methionine

- Alcohol also causes impaired hepatic metabolism of methionine, S-adenosylmethionine, and folate. This causes **decreased levels of glutathione** and **sensitizes the liver to oxidative injury**.

Malnutrition and Deficiencies of Vitamins

- When alcohol becomes a major source of calories in the diet of an alcoholic, the individual may develop malnutrition and vitamin deficiencies (such as thiamine). Additional factors, such as by impaired digestive function, (due to chronic gastric and intestinal mucosal damage and pancreatitis) may further contribute these defects.

Induction of Enzymes

- **CYP2E1** and other cytochrome P-450 enzymes in the liver are induced by alcohol and increases alcohol catabolism in the endoplasmic reticulum. When alcohol concentration in the blood is high, it competes with other compounds metabolized by the same enzyme system. This increases the **conversion of other compounds like drugs** (e.g. acetaminophen) to **toxic metabolites**.

Mechanisms of Fibrosis/Cirrhosis (Fig. 19.14)

- **Stellate cells** (Ito cells or perisinusoidal cells) are present in the space of Disse between hepatocytes and sinusoidal endothelial cells. Normally, the stellate cells are quiescent and store vitamin A.

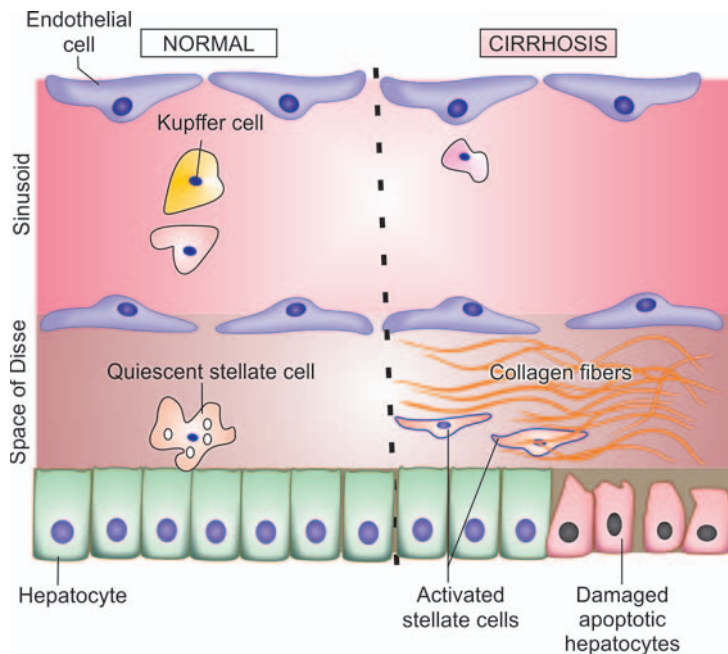
Activation of Stellate Cells

- **One of the characteristic features of cirrhosis is fibrosis.** Alcohol activates hepatic stellate cell → **transformed into highly fibrogenic cells with myofibroblast-like contractile property** → produce collagen (fibrosis) and lose their stored vitamin A. Myofibroblasts are also capable of constricting sinusoidal vascular channels thereby increasing vascular resistance within the liver.
- **Causes of stellate cell activation:**
 - **Cytokine and chemokine:** It is produced by Kupffer cells, endothelial cells, hepatocytes, and bile duct epithelial cells. For example transforming growth factor β (TGF- β).
 - **Inflammatory cytokines:** These are produced by chronic inflammation and include: Tumor necrosis factor (TNF), lymphotoxin, interleukin 1 β (IL-1 β), and lipid peroxidation products.
 - **Oxidative stress.**

Mechanisms of Steatosis in Chronic Alcoholism (refer Fig. 1.13)

Alcohol is a hepatotoxin and **steatosis** is the **reversible** manifestation of chronic alcoholism. Mechanisms by which alcohol causes steatosis of liver are (refer page 16–17):

1. Increases the catabolism of fat in the peripheral tissues (lipolysis) and increases delivery of free fatty acids to the liver. Most of the fat deposited in the liver is derived from the diet.
2. Increases the synthesis of fatty acid in the liver.
3. Decreases the oxidation of fatty acids by mitochondria.
4. Increases the production of triglycerides.
5. Impairs the assembly and secretion/release of lipoproteins.



Stellate (Ito) cells in cirrhosis:

- Produce fibrosis
- Deposits type I and type III collagen in the lobule.

Alcoholic cirrhosis: Activation of stellate cells into myofibroblast-like cells by alcohol is involved in the pathogenesis of fibrosis.

Stellate cells (Ito cells or perisinusoidal cells): Normally quiescent and store vitamin A.

Fig. 19.14: Pathogenesis of fibrosis in cirrhosis of liver. Activation of the stellate cell is followed by proliferation of fibroblasts and the deposition of collagen in the space of Disse

MORPHOLOGY

Hepatic Steatosis (Fatty Liver)

Gross: The liver is **enlarged** (can cause massive enlargement of the liver and may weigh about 4–6 kg), **soft, yellow and greasy** (refer Fig. 1.14).

Microscopy (Figs 19.15 and 1.15)

1. **Microvesicular steatosis:** It is the accumulation of small, clear vacuoles of lipid within the cytoplasm of hepatocytes. It is the initial and most common histologic response. It affects **acinar zone 3 (perivenular/centrilobular region of lobule)** where alcohol dehydrogenase (the major enzyme responsible for alcohol metabolism) is located and hence, affected first. The nuclei of affected hepatocytes are centrally located and cytoplasm looks foamy.
2. **Macrovesicular steatosis:** It develops with continuing alcohol ingestion. This creates clear large, lipid vacuole/s (single or multiple), which compress and displace the hepatocyte nucleus to the periphery of the cell. However, with the cessation of alcohol drinking fatty change are completely reversible and liver returns to normal.
3. **Absence of inflammation or fibrosis:** There is usually neither inflammation nor fibrosis. But continued alcohol intake leads to fibrosis around the terminal hepatic veins (perivenular) and adjacent to sinusoids.

Hepatic steatosis:

- Microvesicular
- Macrovesicular
- No inflammation
- No fibrosis.

Panlobular micro and macrovesicular steatosis indicates alcohol as etiology.

Alcoholic Hepatitis (Alcoholic Steatohepatitis)

Q. Write short note on morphology of alcoholic hepatitis.

Alcoholic hepatitis may be a precursor to the development of cirrhosis.

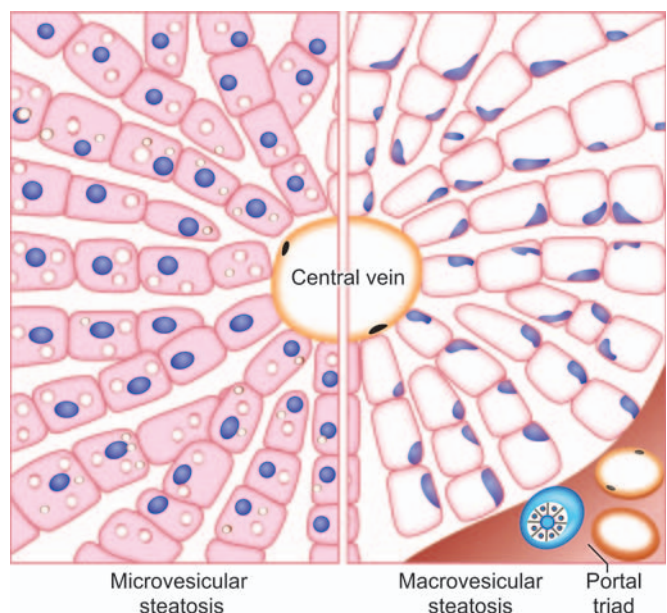


Fig. 19.15: Hepatic steatosis. Diagrammatic appearance of microvesicular (left half) and macrovesicular steatosis (right half)

MORPHOLOGY**Gross**

The liver may be **enlarged**; **yellow** due to steatosis and **firm** due to increased fibrosis.

Microscopy (Fig. 19.16)

Alcoholic hepatitis has four characteristic features and the lesions are predominantly centrilobular.

1. **Ballooning degeneration of hepatocyte:** It is characterized by **swollen liver cells** (hepatocytes) having pale-stained, finely granular or clumped cytoplasm. This is **due to accumulation of fat, water and proteins**. It is predominant in centrilobular region (zone 3 of acinus). Severe ballooning degeneration may lead to → hepatocyte necrosis.
2. **Mallory bodies (Mallory–Denk bodies/Mallory hyaline):** They consist of **tangled skeins of cytokeratin intermediate filaments** (such as cytokeratin 8 and 18). They appear as **dense, eosinophilic ropey cytoplasmic inclusions/clumps**, usually situated in a **perinuclear** location, in the degenerating hepatocytes (Figs 19.16 and refer Fig. 1.28A). Mallory bodies are a **characteristic but not specific** feature of alcoholic liver disease.
3. **Neutrophilic infiltration:** They are commonly seen **around ballooned hepatocytes**, particularly those containing Mallory bodies. The **portal tracts** may be infiltrated by **lymphocytes and macrophages** but is not a prominent feature.
4. **Alcoholic steatofibrosis:** Alcoholic hepatitis may activate sinusoidal stellate cells and portal fibroblasts. This produces fibrosis which initially starts as sclerosis of central veins followed by perisinusoidal fibrosis in the space of Disse of the centrilobular region. The fibrosis spreads further outward to the periphery of lobule, enclosing single or small groups of hepatocytes in a chicken wire fence pattern. In late stages, fibrosis may link fibrous tissue from central vein to portal tracts. Eventually, nodularity, may develop leading to cirrhosis.

5. **Variable degree of steatosis:** Described earlier under steatosis.

Alcoholic hepatitis:

1. Ballooning degeneration
2. Mallory bodies
3. Neutrophilic infiltration
4. Steatosis
5. Perivenular fibrosis.

Mallory bodies: Seen in

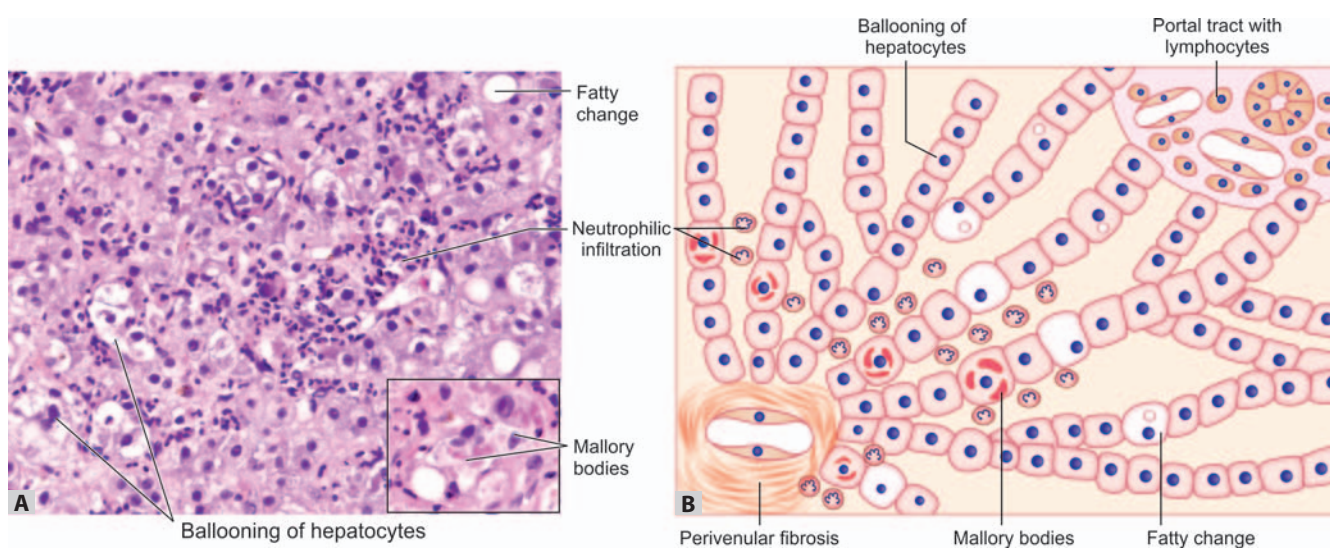
1. Alcoholic hepatitis
2. Nonalcoholic fatty liver disease (NAFLD)
3. Primary biliary cirrhosis (PBC)
4. Wilson disease
5. Chronic cholestatic syndromes
6. Hepatocellular tumors.

Mallory bodies: Tangled skeins of cytokeratin intermediate filaments (such as cytokeratin 8 and 18).

Features of alcoholic cirrhosis are discussed below.

Alcoholic Cirrhosis**Q. Write short note on morphology of alcoholic cirrhosis.**

- Cirrhosis is **chronic, irreversible** and **end-stage** of alcoholic liver disease. It is characterized by diffuse loss of architecture with fibrosis and parenchymal nodular regeneration. Usually evolves slowly and insidiously. It is also known as **Laennec cirrhosis, portal cirrhosis and nutritional cirrhosis**.



Figs 19.16A and B: Alcoholic hepatitis showing ballooning degeneration of hepatocytes, fatty change, Mallory bodies and neutrophilic infiltration: (A) Photomicrograph and inset shows Mallory bodies; (B) Diagrammatic

MORPHOLOGY

Gross

Early stage: Liver appears **yellow tan, fatty, and enlarged**, usually weighs above 2 kg.

Late stage (Fig. 19.17): Liver appears **brown, nonfatty** (loses fat), **diffusely nodular and firm**. The size of liver progressively becomes **smaller than normal (shrunk)**. The weight may be sometimes less than 1 kg.

- **Capsular surface** of liver is **nodular** or may have a pig-skin texture.
- As it advances, the nodularity becomes more prominent and nodules are less than 3 mm (**micronodular**) → “**hobnail**” appearance on the surface of the liver.
- With time, the nodules may coalesce to form larger nodules and forms a **mixed micronodular and macronodular pattern**.
- End-stage alcoholic cirrhosis resemble cirrhosis due to other causes and the underlying etiology cannot be determined by both gross and microscopic examination.

Microscopy (Fig. 19.18)

Alcoholic cirrhosis:

1. Loss of architecture
 2. Fibrosis
 3. Regenerating nodules.
1. **Loss of architecture:** The hepatocyte injury and consequent fibrosis involves the entire liver → diffuse loss of the architecture.
 2. **Regenerating nodules:**
 - The liver cell damage and fibrosis → stimulate the surviving hepatocytes to regenerate and proliferate → form regenerating nodules surrounded by fibrous septa. The regeneration nodules lack the normal structure of the liver lobules or acini.

- Initially, the regenerating nodules are uniform with diameters less than 0.3 cm and are called as **micronodules**.
- Later wider bands of fibrosis create nodules larger than 0.3 cm and are called **macronodules**.
- Finally, the liver shows **mixed micronodular and macronodular pattern**. Mallory bodies are usually **not** seen at this stage.

3. **Fibrosis:** It is initially delicate and extend through sinusoids from **central-to-portal regions** as well as from **portal tract to portal tract**. Later there are **broad bands of fibrosis**.

4. **Vascular reorganization:** The parenchymal damage and fibrosis disrupt the vascular architecture of the liver. New vascular channels will be formed in the fibrotic septa, which connect the vessels in the portal region (hepatic arteries and portal veins) to terminal hepatic veins, shunting blood from the parenchyma.

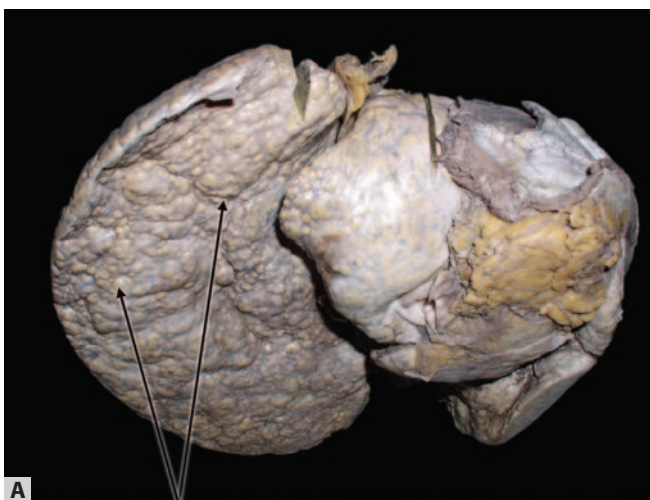
Clinical Features

Hepatic Steatosis (Fatty Liver)

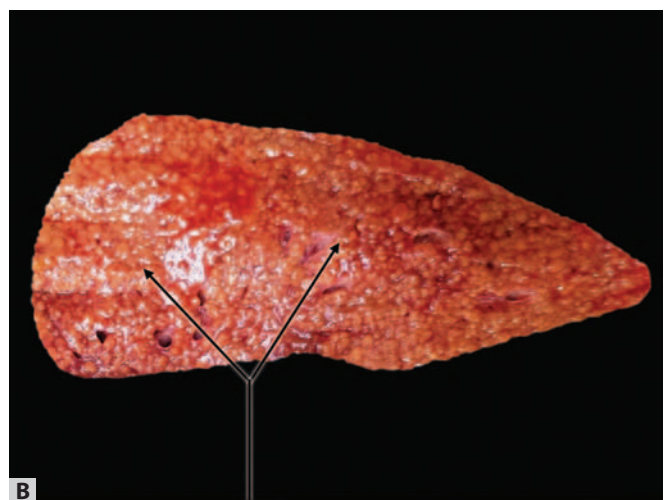
- It may produce hepatomegaly and there may be mild elevation of serum bilirubin, alkaline phosphatase and γ -glutamyl transpeptidase (GGTP). Alcohol withdrawal and with the provision of an adequate diet, the liver can return to normal.

Alcoholic Hepatitis

- It appears acutely, usually following a bout of heavy drinking. Symptoms may be nonspecific, such as malaise, anorexia, weight loss, upper abdominal discomfort and tender hepatomegaly. Repeated bouts of alcoholic hepatitis may lead to cirrhosis in about one-third of

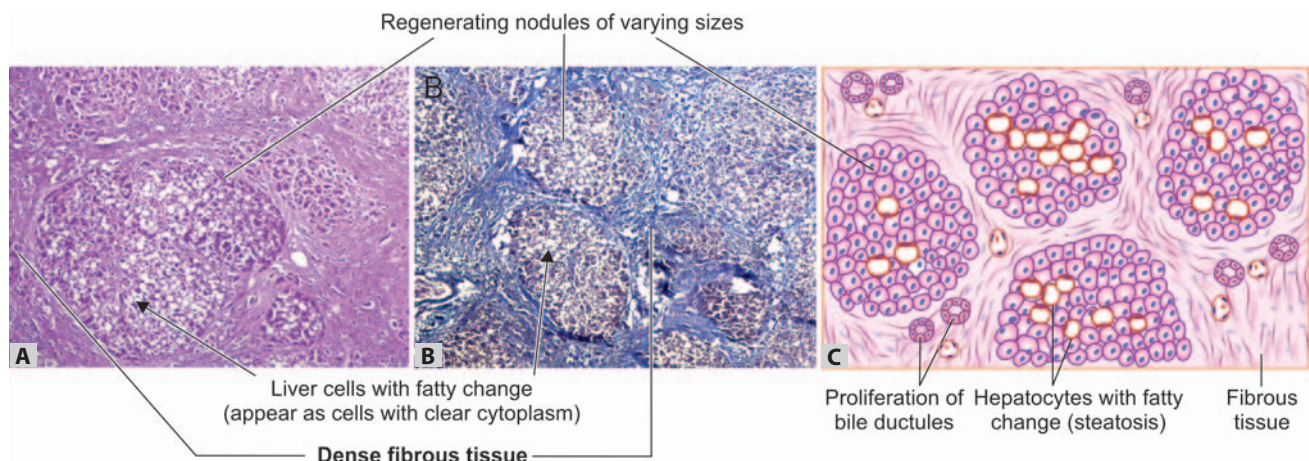


Outer aspect of liver showing multiple nodules



Cut section showing shrunken liver replaced by nodules

Figs 19.17A and B: Gross appearance of alcoholic cirrhosis showing diffuse replacement of parenchyma by micronodules: (A) Outer aspect; (B) Cut section



Figs 19.18A to C: Microscopic appearance of alcoholic cirrhosis: (A) Photomicrograph; (B) Masson trichrome; (C) Diagrammatic showing abnormal regenerating nodules of varying sizes separated by fibrous tissue. In B, fibrous tissue appears blue (Masson trichrome)

patients within a few years. Alcoholic hepatitis may be superimposed on cirrhosis.

Alcoholic Cirrhosis

Symptoms are similar to other forms of cirrhosis (refer page 547).

Laboratory Diagnosis of Alcoholic Liver Disease

AST/ALT >2 is highly suggestive of alcohol as the cause of liver disease.

- Elevated AST which is more than ALT.
- Raised gamma glutamyl transpeptidase.
- Hyperbilirubinemia.
- Raised serum alkaline phosphatase.
- Hypoproteinemia with reversal of albumin–globulin ratio.
- Prolonged prothrombin and partial thromboplastin time.
- Anemia.
- Neutrophilic leukocytosis in alcoholic hepatitis.

Causes of death in alcoholic liver disease

- Hepatic coma
- Massive gastrointestinal hemorrhage
- Intercurrent infection
- Hepatorenal syndrome
- Hepatocellular carcinoma.

CIRRHOSIS

Cirrhosis: End-stage process with

- Bridging fibrous septa
- Most or entire liver involvement
- Regenerating nodules consisting of senescent and replicating cells.

Definition: Cirrhosis is an **end stage** of any chronic liver disease. It is a **diffuse process** (entire liver is involved) characterized by **fibrosis** and conversion of normal architecture to structurally **abnormal regenerating nodules** of liver cells.

Cirrhosis: Difficult to identify the cause on morphological features alone, because all advanced cases of cirrhosis tends to have a similar appearance.

Morphological Characteristics

The three main morphologic characteristics of cirrhosis are as follows:

Fibrosis

- It is the characteristic feature of progressive liver damage. The fibrous tissue form delicate bands or broad scars and link portal tracts with one another and portal tracts with terminal hepatic veins.

Regenerating Nodules

- Liver cell damage is compensated by regeneration of hepatocytes. These regenerating hepatocytes forms nodules and are surrounded by fibrosis. Nodularity results from cycles of hepatocyte regeneration and scarring. The regenerating liver cells does not maintain the normal architecture. The size of nodules vary from very small (<0.3 cm, micronodules) to large (several centimeters, macronodules).

Loss of Architecture

- The hepatocyte injury and consequent fibrosis are diffuse processes, which occur in the entire liver. This disrupts the architecture of the **entire liver**.

Classification

Classification of cirrhosis:

- Morphological classification
- Etiological classification.

Morphological Classification (Fig. 19.19)

Morphological classification:

- Micronodular
- Macronodular
- Mixed

Depending on the size of the regeneration nodules cirrhosis is classified as micronodular, macronodular and mixed.

Micronodular Cirrhosis

It is characterized by **regular** and **small nodules** measuring **less than 3 mm in diameter**. The **fibrous tissue septa** are **usually thin** and fibrous septa bridge portal tracts and central veins (portal-portal and/portal-central) resulting in a small nodule without central structures (e.g. alcoholic cirrhosis).

Macronodular Cirrhosis

It is characterized by the presence of nodules of variable size, more **irregular** than in the micronodular cirrhosis and usually **larger than 3 mm in diameter**. The **fibrous tissue septa** are **broad** and the nodules are more variable in composition and are often composed of multiple acini. Micronodular cirrhosis can be converted into a macronodular form by continued regeneration and expansion of existing nodules. For example cirrhosis associated with chronic hepatitis. The macronodular cirrhosis has an **increased risk** of developing **carcinoma** of liver.

Mixed Cirrhosis

It consists of both micronodules and some macronodules.

Depending on the activity, each of these forms may sub-classified as an active and inactive form.

- Active form: It is characterized by evidence of continuing liver cell necrosis and inflammatory reaction.
- Inactive form: It shows neither liver cell necrosis nor inflammation.

BOX 19.2: Main causes of cirrhosis

- Alcohol is one of the commonest causes
- Viral hepatitis (HBV and HCV)
- Non-alcoholic steatohepatitis (NASH)
- Hemochromatosis
- Autoimmune liver disease (autoimmune hepatitis and primary biliary cirrhosis)
- Intrahepatic or extrahepatic biliary obstruction: Recurrent biliary obstruction (e.g. gallstones)
- Metabolic disorders: Wilson's disease
- 'Cryptogenic' (hidden cause) or idiopathic
- Drugs and toxins
- Indian childhood cirrhosis.

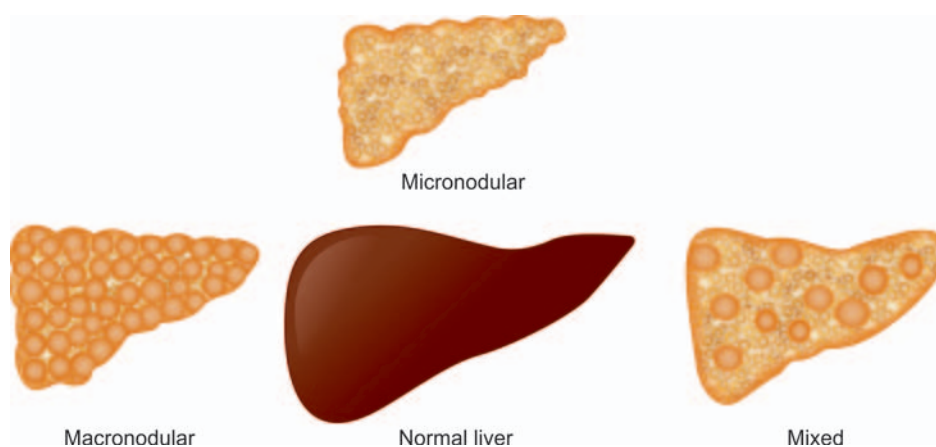


Fig. 19.19: Morphological classification of cirrhosis

Etiological Classification

This classification takes into consideration clinical, biochemical, immunological or biopsy features. Main causes of cirrhosis are shown in Box 19.2.

Pathogenesis

Four important processes are involved:

- **Death of liver cells with loss of architecture:** The pathogenesis of hepatocyte injury varies depending on the etiological agent. The mechanism by which alcohol causes hepatocyte injury is discussed on pages 539-542.
- **Fibrosis (Fig. 19.14):** It is mainly due to the **activation of hepatic stellate cells**, which are transformed into highly fibrogenic cells called myofibroblasts. It is discussed in detail in page 542.
- **Regenerating nodules:** The liver cell damage and fibrosis stimulate the surviving hepatocytes to regenerate and proliferate to form regenerating nodules (refer page 547).
- **Vascular reorganization:** The parenchymal damage and fibrosis disrupt the vascular architecture of the liver (refer pages 549).

The net outcome is formation of a fibrotic and nodular liver. The blood supply to hepatocytes is severely compromised, as well as ability of liver cells to secrete substances into blood.

Clinical Features

The clinical features of cirrhosis range widely:

- Initial phase: It is termed as “**compensated**” cirrhosis, the patient may be asymptomatic.
- Later phase: It is termed as “**decompensated**” cirrhosis, presents with complications of portal hypertension or liver dysfunction (or both).

Nonspecific clinical manifestations: Anorexia, weight loss, weakness, and in advanced disease, symptoms and signs of hepatic failure may develop.

Hepatic failure is usually precipitated by systemic infection or gastrointestinal hemorrhage.

Cause of death in cirrhosis

1. Progressive liver failure
2. Complication related to portal hypertension
3. Development of hepatocellular carcinoma.

PORTAL HYPERTENSION

Portal hypertension in cirrhosis: Due to increased intrahepatic resistance to blood flow through the liver and increase in portal venous inflow.

Portal hypertension is defined as the **elevation of the hepatic venous pressure** above 7 mm Hg.

Causes of Portal Hypertension

The causes of portal hypertension can be divided into three categories:

- **Prehepatic causes:** Obstructive thrombosis of the portal vein before it ramifies within the liver or massive splenomegaly with increased splenic vein blood flow.
- **Posthepatic causes:** E.g. severe right-sided heart failure, constrictive pericarditis, and hepatic vein outflow obstruction.
- **Intrahepatic causes:** E.g. cirrhosis (main cause), schistosomiasis, massive fatty change and diffuse fibrosing granulomatous disease (e.g. sarcoidosis).

Most common cause of portal hypertension in adults: Cirrhosis followed by non-cirrhotic portal fibrosis.

Most common cause of portal hypertension in children: Extrahepatic portal vein obstruction (EHPVO).

Pathogenesis of Portal Hypertension in Cirrhosis

It is produced by a combination of two simultaneously occurring processes:

1. **Increased intrahepatic resistance to blood flow through the liver:**
 - **Deposition of fibrous tissue (scarring):** Fibrosis and compression by regenerative nodules (fixed component) **increases the sinusoidal vascular resistance. In the fibrous septa, anastomosis develop between the arterial and portal system.** These impose arterial pressures on the low pressure portal venous system and contribute to portal hypertension.
 - **Active vasoconstriction: Contraction of vascular smooth muscle cells and myofibroblasts** (functional component) also **increases the sinusoidal vascular resistance.** Intrahepatic vasoconstriction is jointly due to deficiency in intrahepatic nitric oxide (NO) by sinusoidal endothelial cells as well as increased production of the vasoconstrictor ET-1.
2. **Increase in portal venous inflow (flow):** It results from the hyperdynamic circulation/consequences of portal hypertension (Fig. 19.20).

Portal vein hypertension in cirrhosis:

Due to intrasinusoidal hypertension produced by compression caused by regenerating nodules.

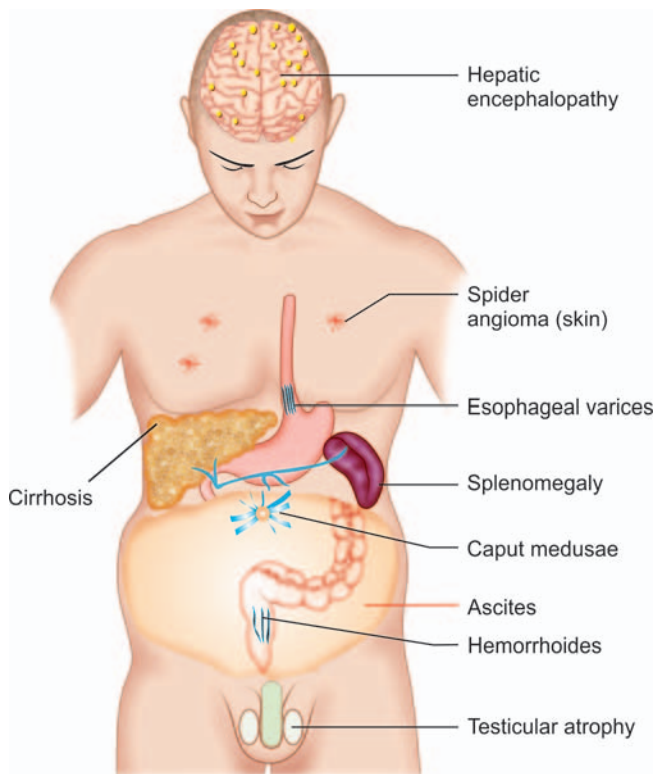


Fig. 19.20: Major clinical consequences of portal hypertension in cirrhosis

Pathogenesis of ascites in cirrhosis:

1. Portal hypertension
2. Hypoalbuminemia
3. Secondary hyperaldosteronism
4. Percolation of hepatic lymph into the peritoneal cavity.

Consequences of Portal Hypertension

- i. Ascites.
- ii. Formation of portosystemic venous shunts.
- iii. Congestive splenomegaly.
- iv. Hepatic encephalopathy.

Ascites

Ascites is defined as the **accumulation of excess fluid** in the **peritoneal cavity**.

The **most common** (85% of cases) **cause** of ascites is **portal hypertension** caused by **cirrhosis**.

Pathogenesis of Ascites in Cirrhosis (Fig. 19.21)

Ascites in cirrhosis: Transudate.

It is complex process and involves the following mechanisms:

- **Portal hypertension:** It increases the hydrostatic pressure in portal vein.

- **Hypoalbuminemia:** It is due to decreased synthetic function in a cirrhotic liver → reduces the plasma oncotic pressure.
- **Splanchnic vasodilation and hyperdynamic circulation.** It reduces systemic arterial blood pressure → activates **renin-angiotensin-aldosterone system** with the development of hyperaldosteronism → leads to **sodium retention** → fluid accumulation and expansion of the extracellular fluid volume.

The combination of portal hypertension, splanchnic arterial vasodilation, and sodium and water retention increases the hydrostatic pressure as well as permeability of interstitial capillaries. It causes extravasation of fluid into the peritoneal cavity.

- **Percolation of hepatic lymph into the peritoneal cavity:** In cirrhosis, hepatic lymphatic flow exceeds thoracic duct capacity. The excess lymph may percolate (pass through liver) into the peritoneal cavity and cause ascites.

Portosystemic Shunts/Varices and Variceal Hemorrhage

- It is one of the complications of portal hypertension due to cirrhosis. In portal hypertension, increased portal vascular resistance leads to increase in the portal system pressure → the blood flow is reversed from portal to systemic circulation by dilation of collateral vessels and development of new vessels. Portosystemic shunting/ bypasses occurs at the sites where the systemic and portal circulation shares common capillary beds.

Main Sites of Portosystemic Shunting/Bypasses

Main sites are (Fig. 19.21):

- **Esophagogastric junction:** These collaterals produce gastroesophageal varices.
- **Veins around and within the rectum:** It results in rectal varices (manifest as hemorrhoids).
- **Retroperitoneum:** Collaterals may form in the retroperitoneum, especially in females and communicate between the ovarian vessels and iliac veins.
- **Umbilicus:** Produce prominent collaterals around the umbilicus. They appear as dilated subcutaneous veins extending from the umbilicus toward the rib margins (**caput medusae**).

Splenomegaly and Hypersplenism

- Portal hypertension is associated with long-standing congestion and may cause **congestive splenomegaly**. The spleen is enlarged and varies in size. Massive splenomegaly may give rise to the syndrome of **hypersplenism**. Hypersplenism is characterized by a decrease in the lifespan of all of the formed elements of the

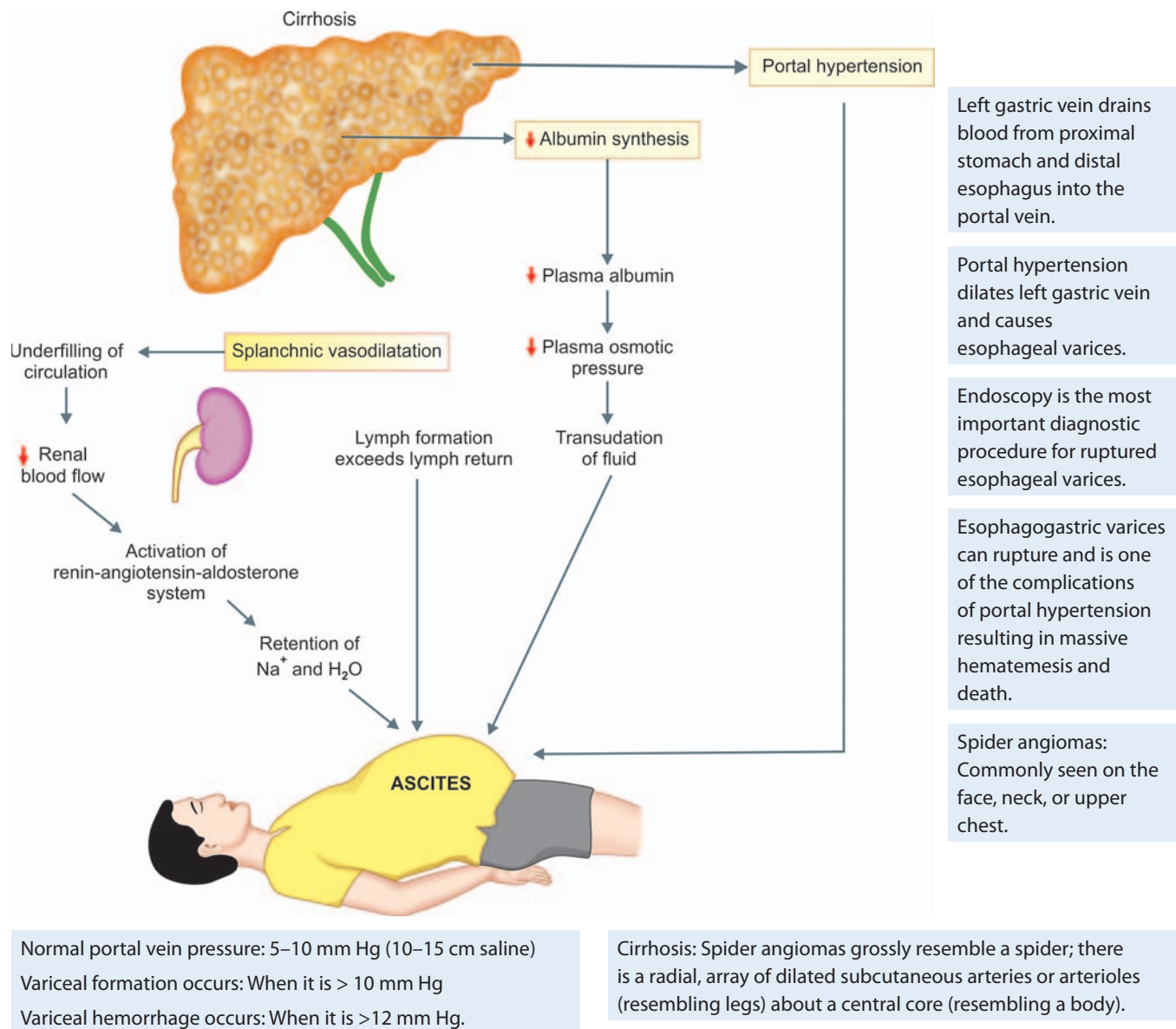


Fig. 19.21: Pathogenesis of ascites in cirrhosis

blood (pancytopenia). **Pancytopenia** of hypersplenism is due to the prolonged transit time of blood through the hyperplastic spleen.

MORPHOLOGY

- **Gross:** The spleen is firm and enlarged; up to 1000 g. Cut surface is uniformly deep red.
- **Microscopy:** The spleen shows dilated sinusoids with thickening of wall due to fibrous tissue. Focal areas of hemorrhages may lead to the formation of **fibrotic, iron-laden nodules**. These are known as **Gamna–Gandy bodies**.

Gamna–Gandy bodies contain:

- Hemosiderin
- Ca^{++} .

Endocrine Complications are Associated with Cirrhosis

It may be either due to the direct effects of alcohol abuse or hepatic dysfunction.

In Men

Cirrhosis: In males produces hyperestrogenism → gynecomastia, spider angioma, and palmar erythema.

Hyperestrogenism

- Chronic liver failure → reduced hepatic catabolism of estrogens + weak androgens are converted to estrogenic

compounds in peripheral tissues → hyperestrogenism → leads to **feminization**.

- The portosystemic shunts secondary to portal hypertension in cirrhosis allow these hormones to bypass the liver.
- Feminization is characterized by **gynecomastia, a female body habitus, and a female distribution of pubic hair**. Hyperestrogenism also causes vascular manifestations, which include **spider angiomas** (upper trunk and face) and **palmar erythema**.

Hypogonadism

- Chronic alcoholics also develop hypogonadism, which is manifested as **testicular atrophy, impotence, and loss of libido**. These are due to direct toxic action of alcohol.

In Women

- They may show features of **gonadal failure**, presenting as oligomenorrhea, amenorrhea, infertility, ovarian atrophy, and loss of secondary sex characteristics. These effects are **due to direct toxic action of alcohol on gonads**.

Main effects of chronic alcoholism are listed in Box 19.3.

BOX 19.3: Main effects of chronic alcoholism

- **Liver:** Fatty liver, alcoholic hepatitis, and cirrhosis (increases the risk of hepatocellular carcinoma)
- **GIT:** Bleeding from gastritis, gastric ulcers and esophageal varices as complication of cirrhosis
- **Others:** Peripheral neuropathy associated with thiamine deficiency, alcoholic cardiomyopathy, and acute and chronic pancreatitis.
- **Major risk factor** for cancers of the oral cavity, larynx, and esophagus.

HEMOCHROMATOSIS

Q. Write short essay/note on hemochromatosis

Hemochromatosis is defined as **excessive accumulation of body iron**.

Classification of Iron Overload

Hemochromatosis may be a primary (hereditary hemochromatosis) or secondary to other acquired or genetic disorders (Box 19.4).

- **Primary (hereditary) hemochromatosis/hemochromatosis:** It is a homozygous-recessive inherited disorder due to excessive absorption of iron.

- **Secondary (acquired) hemochromatosis/hemosiderosis:** Accumulation of iron in tissues may occur secondary to other disorders.

BOX 19.4: Classification of iron overload

1. Hereditary hemochromatosis

- Mutations of genes encoding HFE, transferrin receptor 2 (TfR2), or hepcidin
- Mutations of genes encoding HJV (hemojuvelin: Juvenile hemochromatosis)

2. Hemosiderosis (secondary hemochromatosis)

- Parenteral iron overload: Transfusions, long-term hemodialysis, aplastic anemia, sickle cell disease
- Ineffective erythropoiesis with increased erythroid activity: β -thalassemia, sideroblastic anemia
- Increased oral intake of iron
- Chronic liver disease: Chronic alcoholic liver disease.

Hemosiderosis: Acquired iron overload.

Pathogenesis

Hemochromatosis: Mutations in HJV, TfR2 and HFE gene which leads to absence of hepcidin.

In hemochromatosis there may be **mutations in HJV, TfR2 and HFE**, which leads to absence of hepcidin → leads to absorption of iron even when there is substantial **elevation of body iron stores** → leads to accumulation of iron mainly in the liver. Symptoms develop usually when the stored iron exceeds 20 g.

Mechanism of Tissue Damage

Hemochromatosis: Iron damages by synthesis of hydroxyl-free radicals.

Excessive iron causes tissue damage by the following mechanisms:

1. **Free radical formation:** It damages the tissue by lipid peroxidation.
2. **Activation of hepatic stellate cells:** It stimulates collagen formation.
3. **Interaction of reactive oxygen species and of iron with DNA:** It results in lethal cell injury or predisposition to hepatocellular carcinoma.

Hemochromatosis: The actions of iron on cells are reversible and if damage is not severe, removal of excess iron with therapy promotes recovery of tissue function.

Organ/Tissues Involved

Hemosiderin gets deposited in the following organs (in decreasing order of severity): **Liver, pancreas, myocardium**, pituitary gland, adrenal gland, thyroid and parathyroid glands, joints, and skin. Mainly it presents with **cirrhosis** and **pancreatic fibrosis**.

Morphology of Liver

MORPHOLOGY

Gross

- In early stages, liver may appear grossly normal or slightly darker in color. With progressive accumulation of iron, the liver (other organs, such as the pancreas) become **chocolate-brown color**. **Cirrhosis** due to hemochromatosis is **initially micronodular**, and **later** become **macronodular** cirrhosis.

Microscopy

- Iron deposits first appear as **finely granular golden-yellow pigment in the cytoplasm** of periportal hepatocytes. Hemosiderin is easily recognized with **Prussian blue stain**, which stains them blue. As iron continues to accumulate, iron accumulates in hepatocytes throughout the lobule, within the bile duct epithelium and Kupffer cells. Fibrosis develops slowly. Initially fibrosis develops at the periportal region, later forms portal-portal bridging fibrosis and leads to micronodular cirrhosis.

Laboratory findings in hemochromatosis:

- Raised serum iron
- Raised % saturation
- Raised serum ferritin
- Reduced total iron binding capacity (TIBC).

Clinical Features

Hemochromatosis: Micronodular cirrhosis, diabetes mellitus and brown skin pigmentation—**bronze diabetes**.

- Excessive iron accumulation is a **slow and progressive process** and symptoms usually develop during the fifth to sixth decades of life. Males are more affected than females (5 to 7:1).
- Classic triad is characterized by
 - a. **Micronodular cirrhosis** in all patients
 - b. **Diabetes mellitus** (75–80%) → bronze diabetes
 - c. **Skin pigmentation** (75–80%).

Death may result from cirrhosis, cardiac disease or hepatocellular carcinoma.

WILSON'S DISEASE

Wilson disease: Reduced incorporation of copper into ceruloplasmin and failure to excrete copper into the bile.

- Wilson's disease is an **autosomal recessive disorder of copper metabolism**, leads to progressive accumulation of toxic levels of copper in the liver and various other tissues. **Copper causes tissue toxicity** and end-organ damage principally the **liver, brain, and eye**.

Ceruloplasmin: Enzyme synthesized in the liver that contains copper.

Etiology

- Major method of elimination of copper from the body is by excretion in the bile and is regulated by Wilson's disease gene, **ATP7B**.
- **In Wilson's disease → mutation of the ATP7B gene → causes deficiency in the ATP7B protein → failure to excrete copper in bile → copper accumulates within the liver → cause toxic liver injury through the ROS produced by the Fenton reaction.**

Wilson disease: Accumulation of copper due to mutation of the metal ion transporter ATP7B gene.

Organs Involved

Liver and extrahepatic sites include: Central nervous system, kidneys, endocrine organs, heart, and musculoskeletal system.

MORPHOLOGY

Liver

It is the main organ involved.

Gross

In early stages, the liver may be grossly normal or show mild degree of steatosis. In later stages, progressive fibrosis leads to **macronodular cirrhosis**.

Microscopy

Wilson's disease: Copper is not visible on routine H and E stains. May be demonstrated by special stains, such as rhodanine or rubeanic acid.

In early Wilson's disease, the changes may be mild. The changes may be as follows:

- **Fatty change (steatosis):** Mild to moderate steatosis is common and may be microvesicular or macrovesicular.

- **Acute hepatitis:** It is similar to viral hepatitis, except accompanying **fatty change**.
- **Chronic hepatitis:** It is common.
- **Other features include:** Hepatocyte necrosis, macrovesicular steatosis, vacuolated hepatocellular nuclei, and **Mallory bodies**. As the **disease progresses**, **periportal fibrosis** may develop and progresses to bridging fibrosis and cirrhosis.
- **Massive liver necrosis:** It is rare and is similar to that caused by viruses or drugs.

Brain

Affects the **basal ganglia**, particularly the **putamen** and shows **atrophy**.

Eye Lesions

Kayser–Fleischer rings are formed **due to green-to-brown deposits of copper in Descemet’s membrane** (in the limbus) of the cornea.

Kayser–Fleischer ring: Green-to-brown deposits of copper in Descemet’s membrane of the cornea.

Clinical Features

- **Age at onset:** It ranges between 6 and 40 years of age.
- **Liver involvement:** It can produce **acute or chronic liver disease**.
- **Neuropsychiatric manifestations:** These include: Mild behavioral changes, frank psychosis, or a **Parkinson disease–like syndrome** (such as tremor).

Biochemical Findings

- **Decreased serum ceruloplasmin.**
- **Increased copper content in liver** (the most sensitive and accurate test).
- **Increased urinary excretion of copper** (the most specific screening test).
- **Serum copper levels:** It is **not useful for diagnosis**, since they may be low, normal, or elevated, depending on the stage of the disease.

BILIARY CIRRHOSIS

Classification

1. Secondary biliary cirrhosis: It develops due to prolonged obstruction of the extrahepatic biliary tree.
2. Primary biliary cirrhosis: It is probably an autoimmune disorder of the intrahepatic biliary tree.

Secondary Biliary Cirrhosis

Definition: Cirrhosis developing secondary to prolonged obstruction of the extrahepatic biliary tree.

Etiology

- **In adults:** **Extrahepatic cholelithiasis** (gallstones), **malignant tumors of the biliary tree or head of the pancreas**, and **strictures** due to previous surgical procedures.
- **In children:** **Biliary atresia**, **cystic fibrosis**, **choledochal cysts** (a cystic anomaly of the extrahepatic biliary tree), and paucity of bile duct syndromes (insufficient intrahepatic bile ducts).

MORPHOLOGY

Gross

Liver is **nodular** and shows **yellow-green pigmentation**. **Hard to cut** and appear **finely granular**.

Microscopy

- **Loss of normal architecture** of the liver.
- Coarse **fibrous septa** subdivide the liver. Under **low power**, it produces a characteristic of irregular **“jigsaw puzzle piece” nodules**.
- Fibrous septa shows **distended small and large bile ducts**, which contain **inlaminated pigmented material**.
- **Extensive proliferation of smaller bile ductules** is seen mainly at the interface between septa in former portal tracts and the parenchyma.
- **Liver cells** may show extensive **feathery degeneration** and formation of **bile lakes**.
- Obstruction favors ascending bacterial infection and associated with neutrophilic infiltration of bile ducts; severe **pylephlebitis** and may lead to abscesses.

Secondary biliary cirrhosis:

- Jigsaw puzzle piece nodules
- Small and large bile ducts distended with bile
- Proliferation of bile ductules
- Bile lakes.

Primary Biliary Cirrhosis (PBC)

Definition: PBC is a **progressive chronic autoimmune liver disease** characterized by **nonsuppurative, inflammatory destruction of intrahepatic bile ducts** (cholangitis).

- **Gender:** It usually affects **middle-aged women**, with a female to male ratio of more than 6:1.
- **Age:** It may occur between 20 and 80 years of age, with peak incidence between **40 and 50 years of age**.

Etiology and Pathogenesis

Primary biliary cirrhosis: Destruction of bile ducts in the portal triad by autoimmune mechanism.

- The exact cause of PBC is not known but immune mechanisms are clearly involved in its pathogenesis. **Genetic and environmental factors play role** in the pathogenesis of the PBC.
- PBC is thought to be an **autoimmune disorder**, but its exact pathogenesis is not known.
- **Mechanism of intrahepatic bile destruction:** Many mechanisms have been proposed and these include:
 - **Aberrant expression of MHC class II** molecules on bile duct epithelial cells
 - **Accumulation of autoreactive T cells** around bile ducts
 - **Antimitochondrial antibodies** to hepatocytes or other antibodies against cellular components (nuclear pore proteins, and centromeric proteins, etc.)
 - The **characteristic autoantibody** detected in PBC is **antimitochondrial antibodies**. They target the E2 component of the pyruvate dehydrogenase complex (PDC-E2). PDC-E2-specific T cells are also detected in these patients, supporting immune-mediated pathogenesis.
- **Consequences of bile duct destruction:** Destruction of bile ducts leads to **impaired secretion of bile, cholestasis, and inflammatory reaction in the portal tract**. This results in **hepatic damage, fibrosis** and ends up in **cirrhosis and liver failure**. Cirrhosis develops several years after the onset of disease and most patients are diagnosed at a pre-cirrhotic stage. Hence, the term cirrhosis is somewhat misleading.

MORPHOLOGY

Gross

- **Early-stage**, PBC may have **few gross findings**, weight may be normal or mildly increased (because of inflammation).
- In **late-stage** PBC, the liver shows uniform and bile stasis stains the **liver green**. Liver **weight is decreased**. The nodules are less than 3 mm in diameter (**micronodular cirrhosis**) and later may show **macronodules**. However, in the **end-stage** it is **not possible** to differentiate it from **secondary biliary cirrhosis** or the **cirrhosis resulting from other causes**.

Microscopy

PBC: Three stages

1. Florid ductal lesion
2. Scarring
3. Cirrhosis.

Three distinct stages:

- **Stage I—florid duct lesion:** It is characterized by **inflammation and injury to bile duct epithelial cells**. The bile ducts are surrounded by a dense collection of **lymphocytes, macrophages and plasma cells**. Lymphocytes may form **lymphoid follicles and few with germinal centers**. **Noncaseating epithelioid granulomas** may be seen in the portal tracts.
- **Stage II—scarring:** **Inflammatory process destroys small bile ducts**. This is accompanied by **proliferation of bile ductules and fibrosis** at the periphery of portal triad → **obstruction to intrahepatic bile flow** → leads to **inflammation, and necrosis** of the adjacent periportal hepatic parenchyma.
- **Stage III—of cirrhosis:** Fibrosis in the portal tract and portal-portal bridging fibrosis lead to cirrhosis.

Laboratory Findings

Primary biliary cirrhosis: Jaundice develops late.

- **Serum alkaline phosphatase** (markers of cholestasis), **γ-glutamyltransferase and cholesterol are raised**.
- Hyperbilirubinemia occurs in late stages.
- **Antimitochondrial antibodies are characteristic and are essential for the diagnosis** of PBC. They are found in 90–95% of patients.

Anti-mitochondrial antibodies are present in about 95% of primary biliary cirrhosis.

Primary biliary cirrhosis: Antimitochondrial antibodies essential for diagnosis.

Clinical Features

Primary biliary cirrhosis: Pruritis before jaundice develops.

- Insidious in onset.
- Commonly present with **pruritus, fatigue, and abdominal discomfort**.
- Other features: These include: Skin pigmentation (due to melanin deposition), eyelid xanthelasmas (cholesterol-rich macrophages), steatorrhea, osteomalacia and/or osteoporosis (due to malabsorption of vitamin D).
- Cirrhotic stage: Jaundice, hepatic decompensation, portal hypertension and variceal bleeding develop.

Prognosis: Increased risk of hepatocellular carcinomas.

PBC: Increased risk of HCC.

Cause of death: Liver failure, massive hemorrhage from esophageal varices and intercurrent infection.

Treatment: Liver transplantation.

LIVER ABSCESSES

General Features

Etiology

- **Echinococcal and amebic infections** and less commonly, by other protozoal and helminthic organisms.
- Bacterial infections in the liver may manifest as pyogenic abscess. They develop as a complication of a bacterial infection elsewhere.

Liver abscess—Route of infection:

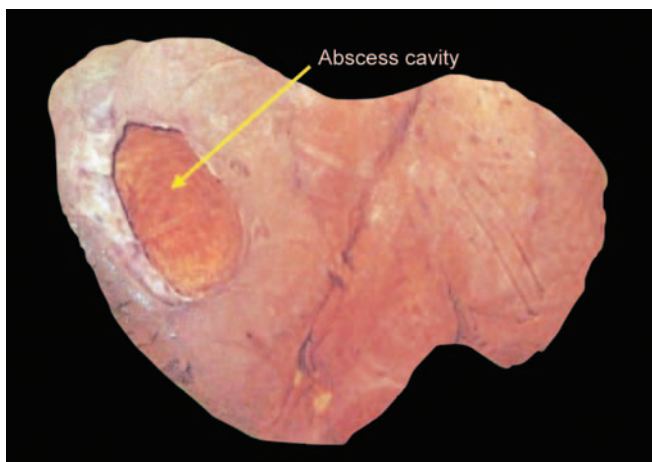
1. Portal vein
2. Arterial blood
3. Ascending infection in the biliary tract
4. Direct invasion.

MORPHOLOGY

- Gross and microscopic appearances are similar to abscesses in other sites and are usually filled with purulent debris. Bacteria may be demonstrated with special stains.

Gross (Fig. 19.22)

- Liver abscesses may be **multiple or solitary**: Bacteremic spread through the arterial or portal system produces multiple small abscesses, whereas direct extension and trauma usually cause solitary large abscesses.
- **More common in the right lobe.**
- Size is variable, ranging up to 10 cm



Liver is the most common site of abdominal visceral abscess.

Pyogenic liver abscess: Accounts for majority of liver abscess.

Most common cause of pyogenic liver abscess:

1. *E.coli*—Western countries
2. *Klebsiella pneumoniae*—Asian countries
3. *Staphylococcus* in children.

Fig. 19.22: Liver with an abscess cavity in the right lobe

Clinical Features

They present with **fever, right upper quadrant pain** and **tender hepatomegaly**. Jaundice may develop when there is extrahepatic biliary obstruction.

Amebic Liver Abscess

Q. Write short note on amebic liver abscess.

- **Most common extraintestinal complication of amebic dysentery** (refer pages 500-501 and Fig. 18.21).

Amebic liver abscess:

- Solitary
- More common in right lobe.

Gross

- Amebic abscess ranges from 8 to 12 cm in diameter and appears well circumscribed. **Amebic abscess are usually located in the subdiaphragmatic region.** The abscess cavity contains thick, dark material that has been likened to **anchovy paste (sause) or chocolate.**

Microscopy

- The trophozoites can be demonstrated in the periphery of the necrotic debris.
- **Clinical features:** The symptoms are similar to pyogenic abscesses. Surgical drainage of large abscesses is important.

Amebic liver abscess: Anchovy paste (sause) or chocolate colored.

• Complications:

- If an amebic abscess continues to grow, it may rupture into the 1) **thoracic cavity to produce empyema or a lung abscess**, 2) may rupture into the **peritoneal cavity**, where it produces **peritonitis**, a complication associated with a mortality rate as high as 40%.
- The amebae may also invade the blood, in which case **abscesses of the brain and lung** may ensue.

MALIGNANT TUMORS OF LIVER

Malignant tumors of liver can be primary or metastatic. Most primary cancers of liver arise from hepatocytes and are termed **hepatocellular carcinoma (HCC)**. Less common are cancers that arise from bile duct known as **cholangiocarcinomas**.

Two rare primary liver cancers are: Hepatoblastomas and angiosarcomas.

Most common benign tumor of liver: Hemangioma.

Angiosarcoma of the liver: Highly aggressive neoplasms, associated with exposure to vinyl chloride (plastic pipes), arsenic, or Thorotrast.

Hepatoblastoma

- Most common liver tumor arising in young childhood.
- **Malignant tumor** and usually fatal.

Hepatoblastoma: Most common primary hepatic tumor of childhood.

MORPHOLOGY

Gross

Appears as a **solitary, large mass** in the **right lobe**.

Microscopy

Two variants

1. **Epithelial type:** It consists of small **polygonal fetal cells** or **smaller embryonal cells**. These tumor cells form **acini, tubules, or papillary structures**.
2. **Mixed epithelial and mesenchymal type:** It is characterized by areas of **both epithelial and mesenchymal differentiation**. The mesenchymal component may be **primitive, mesenchyme** (with spindle or stellate cells with little cytoplasm), or show differentiation towards osteoid, cartilage, or striated muscle.

Hepatocellular Carcinoma (HCC)

- Hepatocellular carcinoma (HCC) is a **malignant tumor derived from hepatocytes or their precursors**.
- **Predominantly in males** with a M:F ratio of 2.4:1.

Etiopathogenesis

Q. Write short note on etiopathogenesis of HCC.

It is multifactorial disease and complex in pathogenesis. It is **probably a multistep process** that involves various risk factors. **Three major and several minor risk factors** are associated with HCC (Table 19.6).

TABLE 19.6: Risk factors for hepatocellular carcinoma

Major risk factors	Minor risk factors
1. Chronic hepatitis: HBV/HCV	1. Hereditary hemochromatosis
2. Alcoholic cirrhosis	2. Wilson's disease
3. Aflatoxin B ₁	3. Tyrosinemia
4. Non-alcoholic steatohepatitis (NASH)	4. α ₁ -Antitrypsin deficiency
	5. Glycogen storage disease
	6. Oral contraceptives
	7. Cigarette smoking

Remember factors as

Major ABCC (A = Aflatoxin, B = HBV, C = HCV, C = Cirrhosis).

Minor HCC (H = Hemochromatosis, C = Cigarette, C = Contraceptive).

HCC: Chronic infection by HBV and HCV most common major risk factors.

Major Risk Factors

1. **Chronic hepatitis:** The risk of liver cancer in individuals infected with **both HCV and HBV is three times higher than with either alone**.
 - **HBV infection:** Chronic HBV infection is strongly associated with HCC, especially **vertical transmission** from infected mothers to her child is a **major risk factor**.
 - **Repeated cycles of liver cell necrosis and regeneration** in chronic hepatitis B is the soil HCC. HBV associated cirrhosis is another major risk factor.
 - The **genome of HBV is integrated** into the **host DNA of liver cells** → may **activate proto-oncogenes into oncogene**, which causes tumor.
 - **X gene of HBV encodes HBV X-protein**, which is a transcriptional activator of many genes. **It can inactivate tumor suppressor genes and cause cell transformation**.
 - **HCV infection:** Most patients with HCV who develop HCC have underlying cirrhosis. **HCV core protein may interact with a many cellular proteins** to cause HCC.
2. **Cirrhosis:** **Strong association between HCC and cirrhosis** and both frequently coexists.
 - **Alcoholic cirrhosis** predisposes to HCC.
 - Male gender, age, and duration of cirrhosis are the major risk factors for HCC in patients with cirrhosis.
3. **Aflatoxin B₁ (chemical carcinogen):** It is a toxin produced by the fungus *Aspergillus flavus*. This fungus **contaminates improperly stored peanuts and grains**. Dietary exposure to aflatoxin B₁ is an important risk factor for hepatocellular carcinoma.
 - **Aflatoxin can bind covalently with cellular DNA of hepatocytes** and cause a specific **mutation of TP53 tumor suppressor gene**.
 - Aflatoxin B₁ and HBV interact synergistically in the pathogenesis of hepatocellular carcinoma.
4. **Non-alcoholic steatohepatitis (NASH)**

HCC: Pre-existing cirrhosis and aflatoxin B₁ are major risk factors.

HCC: Most common primary malignant tumor of liver.

Minor Risk Factors

- These include: Genetic factors, age, gender, chemicals, hormones, and nutrition.
 - **Hemochromatosis:** Excessive free iron may be carcinogenic and generates mutagenic reactive oxygen species.
 - **Wilson's disease:** It is characterized by accumulation of copper in the liver.
 - **Metabolic disorders:**
 - ♦ With cirrhosis: Inherited metabolic disorders complicated by cirrhosis. Examples, α_1 -antitrypsin deficiency and type 1 hereditary tyrosinemia.
 - ♦ Without cirrhosis: Certain inherited diseases in the absence of cirrhosis. Example, type 1 glycogen storage disease.
 - **Oral contraceptive steroids.**
 - **Cigarette smoking.**

Molecular Basis of HCC

Both genetic and epigenetic alterations have been detected in hepatocellular carcinoma.

Genetic Alterations

These include aneuploidy, point mutations, and both loss and gain of chromosomal components.

- **Mutation in tumor DNA repair genes:** Chronic hepatitis of any cause (viruses, alcohol, and metabolic or autoimmune) leads to repeated cycles of liver cell death, regeneration, and repair → may lead to many mutations in DNA repair genes.
- **Activation of oncogene:** Point mutations in cellular proto-oncogenes, such as *KRAS* may result in oncogene. Others include: **Overexpression of growth factor TGF- α , point mutation or overexpression of WNT signal transduction proto-oncogenes β -catenin.** About 50% of HCC cases are associated with activation of WNT or AKT pathways. X protein of the hepatitis B virus have been shown to have oncogenic effects.
- **Inactivation of tumor suppressor gene:** Integration of HBV genome into host hepatocyte genomic DNA may cause inactivation of tumor suppressor genes (e.g. *TP53*).

Epigenetic Alterations

- a. **c-MYC amplification** by epigenetic alterations.
- b. **Telomerase:** Cells may become immortal, through activation of the cellular telomerase enzyme.

One of the concepts is that some HCCs may arise from epithelial stem cells of liver.

Precursor lesions of HCC

1. **Hepatocellular adenoma** particularly those with β -catenin activating mutations.
2. **Cellular dysplasias in chronic liver disease:** May be seen in chronic liver disease, before or after development of cirrhosis. These include:
 - **Small-cell change:** is probably premalignant. These liver cells have high nuclear-cytoplasmic ratio and mild nuclear hyperchromasia and/or pleomorphism.
 - **Large-cell change:** is a marker of increased risk of HCC in the liver and in hepatitis B. They may also be directly premalignant. These cells larger than normal liver cells having large, multiple, moderately pleomorphic nuclei with normal nuclear-cytoplasmic ratio.
3. **Dysplastic nodules:** These are nodules having different appearance than cirrhotic nodules, that are usually detected radiologically or in resected specimens of cirrhosis.
 - **Low-grade dysplastic nodules:** They may or may not transform to higher grade lesions, but they are indicator of higher risk for HCC. They don't have cytological or architectural atypia,
 - **High-grade dysplastic nodules:** Important precursors of HCC in viral hepatitis and alcoholic liver disease. The cells of these nodules have cytological (e.g., small-cell change) or architectural features suggestive of, but not sufficient for diagnosis of frank HCC.

MORPHOLOGY

HCC: Bile production by neoplastic cells is hallmark.

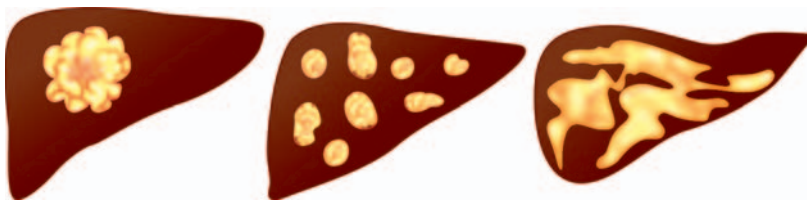
Gross (Fig. 19.23)

Q. Write short note on morphology of HCC.

Three patterns. All patterns may cause enlargement of liver, particularly the large unifocal and multinodular patterns. **Areas of necrosis and hemorrhage** are common in all patterns.

1. **Unifocal** (usually large): Tumor appears as **large** circumscribed **single mass** in a portion of the liver (Fig. 19.24).
2. **Multifocal:** This pattern shows **multiple nodules** of variable size which are widely distributed.
3. **Diffusely infiltrative:** This type is characterized by **large part of the liver or sometimes entire liver infiltrated by homogeneous indistinct tumor nodules.** The tumor may blend into a cirrhotic liver background and may be difficult to differentiate from the regenerating nodules of cirrhosis.

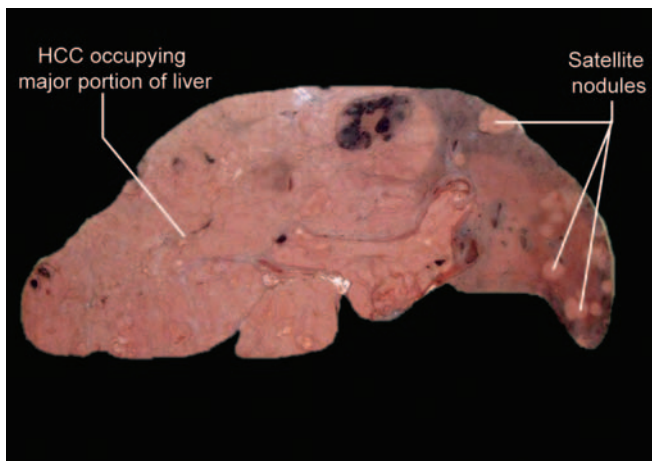
Color: HCCs are usually **light brown, yellowish-white or gray** in color. **Production of bile** by tumor cells may cause **greenish-brown discoloration** of the tumor.



Lectin fraction -3 of AFP (AFP-I3):

- Highly specific to HCC
- Indicator of poorly-differentiated HCC
- Unfavorable prognosis.

Figs 19.23A to C: Gross patterns of hepatocellular carcinoma: (A) Unifocal; (B) Multifocal, (C) Diffuse infiltrative



Hep-par 1: Used in diagnosing hepatocellular carcinoma.

HCC gross: Three patterns

1. Unifocal
2. Multifocal
3. Diffusely infiltrative.

Hep-par 1 (hepatocyte paraffin 1): Specific for hepatocyte mitochondria and is considered the most specific and sensitive marker of normal and neoplastic hepatocytes.

Fig. 19.24: Hepatocellular carcinoma, liver with a large gray-white tumor occupying the major portion of liver along with satellite nodules

Microscopy (Fig. 19.25)

HCC: Tumor cells recapitulate varying degrees of normal liver architecture.

HCC graded as well-differentiated, moderately differentiated, and undifferentiated (pleomorphic) forms.

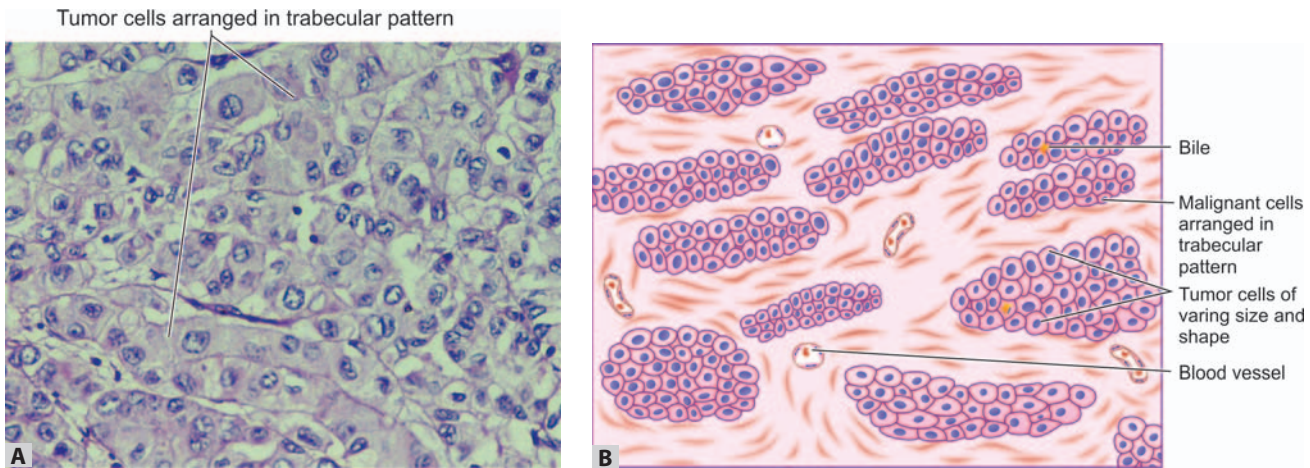
1. **Well-differentiated HCC:** Tumor cells can be recognizable as hepatocytic in origin. Bile production by tumor cells is the **hallmark** of hepatocellular carcinoma. Tumor cells are arranged in trabecular and acinar (pseudoglandular) pattern.
 - **Trabecular pattern:** It shows malignant hepatocytes arranged in trabeculae (**several layers of malignant cells**) or **irregular anastomosing plates**. The tumor cells are **polygonal** and have **abundant, slightly granular cytoplasm**. The nuclei are **large and hyperchromatic** and show **prominent nucleoli**.
 - **Acinar, pseudoglandular pattern (adenoid):** In this pattern, malignant hepatocytes are arranged around a **lumen and resemble glands**. The lumen may contain bile. The acini formed by the tumor cells are not true glands, hence the name pseudoglandular.
2. **Moderately differentiated HCC:** This grade may show solid, scirrhous, and clear-cell pattern.
 - **Solid variety:** The tumor cells usually are small and may show considerable variation in shape. Bile production is rare.
 - **Scirrhous variety:** Malignant cells are arranged in narrow bundles separated by **abundant fibrous stroma**.

- **Clear cell variety:** It consists of predominantly or exclusively clear cells. The clear cytoplasm is due to glycogen or, in some cases due to fat.
- 3. **Poorly or undifferentiated HCC:** It consists of **pleomorphic cells with great variation in size and shape**. The nuclei also are extremely variable in size and shape. Many bizarre-looking anaplastic giant cells can be seen.

Globular hyaline structures may be seen in the cytoplasm of all types of hepatocellular carcinoma. They represent **alpha-fetoprotein, α_1 -antitrypsin**, or other proteins. **Mallory's hyaline** may be occasionally seen.

Spread

- **Local spread:** HCC may first spread within the liver itself and develop satellite nodules. **Intrahepatic metastases (by vascular invasion/direct extension)** more likely to occur when the size of tumors reach 3 cm. Local invasion of the diaphragm is common.
- **Lymphatic spread:** HCC may spread to portal lymph nodes, perihilar, peripancreatic, and para-aortic nodes.
- **Blood spread:**
 - **All patterns of HCCs have a strong tendency for invasion of vessels.**
 - This may result in extensive intrahepatic metastases.
 - The portal vein and its branches are infiltrated by tumor.



Figs 19.25A and B: Hepatocellular carcinoma composed of malignant hepatocytes arranged in trabecular pattern: (A) Photomicrograph; (B) Diagrammatic

- Occasionally, long, snake-like tumor masses may **invade the portal vein** and occlude portal circulation.
- Rarely, tumor may invade inferior vena cava and extend into the right side of the heart through the hepatic veins.
- It may metastasize to the **lungs**.

HCC: Strong tendency for invasion of vessels.

Fibrolamellar HCC

Q. Write short note on fibrolamellar HCC.

- It is a **distinctive uncommon variant** of HCC and constitutes about 5% of HCCs.
- **Age and sex:** It occurs in **young patients without cirrhosis**.
- **Etiology:** Unknown.

Tumor marker for fibrolamellar HCC: Neurotensin.

MORPHOLOGY

Gross

- **Single large, hard “scirrhous” well-circumscribed tumor with central stellate fibrous scar.**

Microscopy

- It consists of **large, polygonal cells with abundant deeply eosinophilic (oncocyctic) cytoplasm and prominent nucleoli**. The tumor cells are arranged in **nests or cords**, and separated by **parallel bands of abundant dense collagen bundles**.
- **Prognosis:** It is **better** than the conventional HCC.

Fibrolamellar HCC:

- Young adults without cirrhosis
- Well circumscribed with central stellate fibrous scar
- Grows slowly
- Better prognosis.

Clinical Features of HCC

- Most patients present with **ill-defined upper abdominal pain, malaise, fatigue and weight loss**.
- On examination, liver appears enlarged, irregular or nodular.

HCC: Associated paraneoplastic syndromes include polycythemia (PTH-related protein), hypoglycemia (insulin-like factor), hypercalcemia and erythrocytosis (EPO-erythropoietin).

Hepatocellular carcinoma: Most common symptom—abdominal pain > weight loss.

Laboratory Findings—Serum Marker

- **Alpha-fetoprotein:** About 50% hepatocellular carcinoma is associated with **high serum levels of alpha-fetoprotein**. However, α -fetoprotein levels are often raised in other neoplastic and non-neoplastic liver diseases and in some extrahepatic disorders.
- **α -L-fucosidase:** It is raised in HCC and also in cirrhosis.
- **Serum des- α -carboxy prothrombin:** It is **raised in a majority of hepatocellular carcinoma**.

HCC: Raised serum AFP.

HCC: Staining for Glypican-3 is used to distinguish early HCC from dysplastic nodules.

Neoplasm with alpha-fetoprotein:

- Hepatocellular carcinoma
- Nonseminomatous germ cell tumors (e.g. endodermal sinus tumor/yolk-sac tumor) of testis.

Non-neoplastic conditions false positive with alpha-fetoprotein:

- Cirrhosis
- Massive liver necrosis
- Chronic hepatitis (especially HCV)
- Normal pregnancy, fetal distress or fetal death, fetal neural tube defects (e.g. anencephaly and spina bifida).

Cause of Death

1. Cachexia.
2. Gastrointestinal or esophageal variceal bleeding.
3. Liver failure with hepatic coma.
4. Rupture of the tumor with fatal hemorrhage (rare).

CHOLANGIOCARCINOMA (CCA)

- It is the **second most common hepatic malignant tumor of liver**.
- **Site:** It may arise anywhere in the biliary tree, from the large intrahepatic bile ducts at the porta hepatis to the smallest bile ductules (within liver).

Risk Factors

- **Primary sclerosing cholangitis (PSC).**
- **Congenital fibropolycystic diseases of the biliary system**, such as Caroli disease and choledochal cysts.
- **HCV infection.**
- Previous **exposure to thorotrast** (formerly used in radiography of the biliary tract).
- **Chronic infection** of the biliary tract by the liver fluke **opisthorchis sinensis**.
- **Premalignant lesions:** Biliary intraepithelial neoplasias (low to high grade, BilIN-1, -2, or -3).

Workers exposed to polyvinyl chloride may develop: Angiosarcoma of liver.

Cholangiocarcinoma of liver—one of the risk factor is: Opisthorchis/Clonorchis sinensis infection.

Classification

CCA is classified according to their location.

- **Intrahepatic** (about 10%).
- **Extrahepatic forms** (about 80–90%)
 - **Perihilar tumors** (50–60% of all CCAs): These are known as **Klatskin tumors** and are located at the **junction of the right and left hepatic ducts** forming the common hepatic duct.

- **Distal bile duct (bile duct carcinomas) tumors** (20–30%): They arise **near the ampulla of Vater**. They **also include periampullary carcinomas**, which consists of adenocarcinoma of the duodenal mucosa and pancreatic carcinoma.

MORPHOLOGY

Klatskin tumors are located at the junction of right and left hepatic ducts. They are the commonest subtype of cholangiocarcinoma.

Gross

- **Extrahepatic CCAs:** These are usually small lesions and appear as **firm, gray nodules within the bile duct wall**.
- **Intrahepatic CCAs:** They develop in the intrahepatic portal tract.

Microscopy

- **Adenocarcinomas:** Well-differentiated adenocarcinomas consist of well-defined **glandular and tubular structures lined by cuboidal to low columnar epithelial cells**.
- **Marked desmoplasia:** It is characterized by dense collagenous stroma separating the glandular structures.

The marker of choice for differentiating HCC and its fibrolamellar variant is AFP.

METASTATIC TUMORS

Q. List four common primary sites of metastatic tumors.

- Metastasis to liver is **more common than primary tumors of liver**. Apart from liver, lungs are also most often involved in the metastatic spread of cancers.
- **Most common site of primary tumor** producing hepatic metastases are the **gastrointestinal tract (colon), breast, lung, and pancreas**. However, any cancer in any site of the body may spread to the liver, including leukemias, melanomas, and lymphomas.

Metastasis to liver: Common primary includes GI tract, breast, lung and pancreas.

MORPHOLOGY

Gross (Fig. 19.26)

- The liver is **enlarged** and may weigh several kilograms.
- The liver may show only **one nodule** or may be completely replaced by **multiple nodules of metastatic deposits**.
- On the surface of the liver, the metastatic **nodules appear as umbilicated masses**.
- **Central umbilication** is due to necrosis (outgrow blood supply) and hemorrhage in the central area of the nodule.

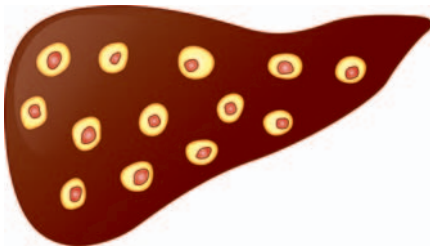


Fig. 19.26: Metastasis to liver showing multiple nodules with umbilication

Microscopic

- The metastatic deposits usually appear **similar to the primary tumor**. Occasionally, they may be so undifferentiated that the primary site cannot be determined.

Most common malignant tumors of liver: Metastatic carcinoma mostly from colon, lung and breast.

Clinical Features

- **Weight loss:** It is a common early finding.
- **Portal hypertension with splenomegaly, ascites, and gastrointestinal bleeding** may occur.
- **Jaundice:** It may be either due to obstruction of the bile ducts or replacement of most of the liver parenchyma.
- Hepatic failure may develop.

Laboratory findings: May show **increase** in the **serum alkaline phosphatase** level.

Prognosis: Most patients die within a year.

GALLBLADDER

ACUTE CHOLECYSTITIS

Functions of gallbladder:

1. Reservoir of bile
2. Concentration of bile
3. Secretion of mucus
4. Acidification of bile.

Gallbladder: Lacks

- Muscularis mucosa
- Submucosa.

Cholecystitis: Inflammation of the gallbladder is known as cholecystitis. It may be acute, chronic, or acute superimposed on chronic.

Common diseases of the gallbladder: Gallstones, acute and chronic cholecystitis.

Acute cholecystitis is a acute **diffuse inflammation** of the gallbladder.

Types

Q. Write short note on cholecystitis.

- **Acute calculous cholecystitis:** It is associated with **gallstone** and is the most common serious complication of gallstone.
- **Acalculous cholecystitis:** It is not associated with gallstones and may occur in severely ill patients.

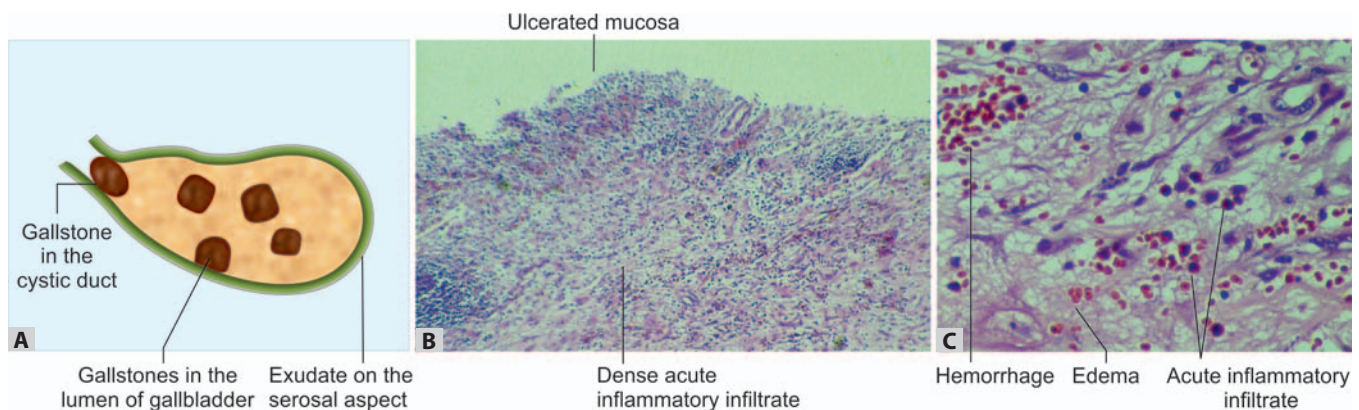
Cholecystitis: Most commonly occurs in association with gallstones.

Pathogenesis

Acute Calculous Cholecystitis

Acute inflammation of the gallbladder develops in **about 90% of cases as a complication due to obstruction of the neck or cystic duct by gallstones**. The inflammation of gallbladder may be brought out by **three factors** namely, 1. chemical inflammation, 2. mechanical inflammation and 3. bacterial inflammation.

1. **Chemical inflammation:** Inflammation of the obstructed gallbladder may be due to chemical irritation.
 - **Production of lysolecithin:** The phospholipase from the epithelium of the gallbladder may act on lecithin present in bile and release **toxic lysolecithin** → can cause chemical inflammation.
 - **Decreased glycoprotein:** Normally, glycoprotein produced by gallbladder mucosa has **protective role**. Its **decreased production** leads to the direct mucosal cell damage by the detergent action of concentrated bile salts.
 - **Supersaturation of bile with cholesterol:** It may cause toxic damage to the epithelium.
 - **Prostaglandins:** These are **released within the wall of the distended gallbladder** and contribute to **inflammation of mucosa and wall of the gallbladder**.
2. **Mechanical inflammation:** **Obstruction of the cystic duct** increases intraluminal pressure and distention of the gallbladder → leads to disturbance of motility (dysmotility) of the gallbladder → results in **obstruction of venous flow and ischemia** of the gallbladder mucosa and wall.



Figs 19.27A to C: (A) Gross features of acute calculous cholecystitis; (B) Microscopy of acute cholecystitis showing ulceration of mucosa; (C) High-power view showing edema, hemorrhage and acute inflammatory infiltrate

3. **Bacterial inflammation:** It may play a role in acute cholecystitis, but **usually bacterial invasion is a secondary event**. The most frequent organisms include *Escherichia coli*, *Klebsiella* species, *Streptococcus* species, and *Clostridium* species.

Pathogenesis of acute calculous cholecystitis:

- Chemical inflammation
- Mechanical inflammation
- Bacterial inflammation.

Acute Acalculous Cholecystitis

- Gallstones are not seen in 5–10% of patients with acute cholecystitis.
- **Risk factors:** Include:
 - Major trauma and burns.
 - Sepsis with hypotension and multisystem organ failure.
 - Immunosuppression.
 - Diabetes mellitus.
 - Nonbiliary major surgical operations.
 - Systemic infections (tuberculosis, syphilis, actinomycosis, etc.).
- **Ischemic injury:** It is the **main pathogenic event** in acute acalculous cholecystitis.
- **Contributing factors:** These include inflammation and edema of the wall-compromising blood flow, dehydration, gallbladder stasis, and accumulation of microcrystals of cholesterol (biliary sludge).

MORPHOLOGY

- Both **acute acalculous** and **calculous cholecystitis** are **morphologically similar**, except that stones are not seen in the acalculous form.

Gross (Fig. 19.27A)

- **Size:** Gallbladder is usually **enlarged, tense and edematous**.
- **Serosa:** It is covered by **fibrinous exudate** and the serosa may appear bright red due to subserosal hemorrhage. In severe cases, serosa may be covered by suppurative exudate.
- **Wall:** It is **thickened** (up to 2 cm) due to edema and hemorrhage.
- **Mucosa:** It is **red or purple** and may show **ulcerations**.
- **Lumen:** In **calculous cholecystitis**, the lumen may contain one or more **stones**. The lumen is filled with turbid fluid.

Microscopy (Figs 19.27B and C)

- Mucosa shows focal **ulcerations**.
- **Wall of the gallbladder** shows **edema, hemorrhage and acute inflammatory cells**. Secondary bacterial infection may lead to suppuration in the gallbladder wall.
- **Widespread necrosis** is found in **gangrenous cholecystitis**.

Complications

1. **Empyema of the gallbladder:** Gallbladder with obstructed cystic duct may be distended with pus (purulent exudate).
2. **Gangrenous cholecystitis:** Gallbladder transformed into a green-black necrotic organ.
3. **Acute gaseous or emphysematous cholecystitis:** It is due to invasion of gas-forming organisms (such as clostridia and coliforms).
4. **Perforation of the gallbladder:** It is due to secondary bacterial infection.
5. **Bile peritonitis:** It may result from the discharge of bile from the distended gallbladder into the peritoneal cavity.
6. **Pericholecystic abscess, abscesses in the liver or abdominal cavity.**

7. **Fistula into the intestine or duodenum:** Perforations may lead to fistula formation with neighboring organs, such as small or large intestine creating a **cholecystenteric fistula**.
8. **Gallstone ileus:** Through the fistula, large gallstone can **pass into the bowel** → result in gallstone ileus or intestinal obstruction.

Clinical Features

Acute cholecystitis:

1. Right upper quadrant pain
2. Fever
3. Leukocytosis.

- Acute cholecystitis presents with **progressive right upper quadrant** or epigastric pain.
- It is frequently associated with **mild fever, anorexia, nausea, and vomiting**. Acute calculous cholecystitis may also **appear suddenly** as an **acute surgical emergency**.
- **Acute acalculous cholecystitis** tends to be **more insidious in onset**.

Laboratory findings: Mild to moderate **leukocytosis** and mild elevations in serum alkaline phosphatase values may be seen.

CHRONIC CHOLECYSTITIS

- **Most common disease of the gallbladder.**
- Associated with **gallstones in ~90%** of the cases.
- May also develop following recurrent attacks of acute cholecystitis.

MORPHOLOGY

- Extremely variable and depends on the severity and the duration of the disease.

Gross (Fig. 19.28)

- **Size:** Gallbladder may be **normal** or may be **shrunk** in severe cases.
- **Serosa:** It is usually **smooth and glistening** but may be dull due to fibrosis.
- **Cut section:** The wall is **thickened, opaque and gray-white**.
- **Lumen:** It usually shows **stones**.

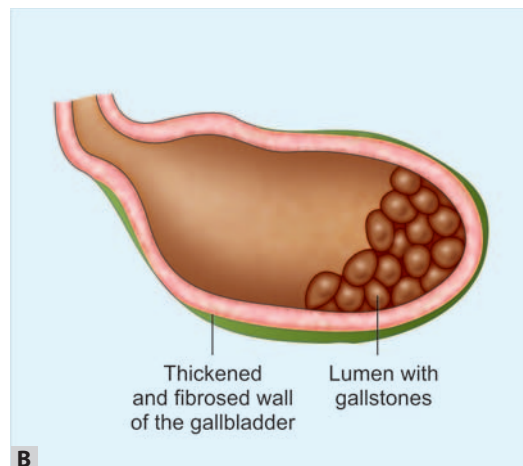
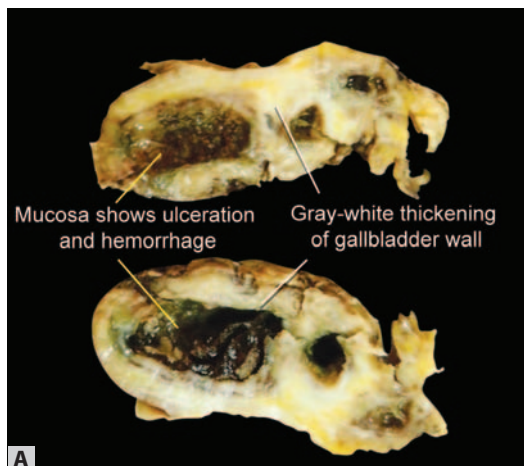
Microscopy (Fig. 19.29)

- **Mild cases:** The inflammation is **scanty** and show **lymphocytes, plasma cells, and macrophages** in the **mucosa and in the subserosal fibrous tissue**.
- **Advanced cases:** It shows **marked subepithelial and subserosal fibrosis**, associated with **mononuclear cell infiltration**.
- **Mucosal epithelium:** It may be **normal or atrophic** or show focal ulceration.
- **Rokitansky–Aschoff sinuses:** These are **irregularly shaped, tubular structures** formed due to **invagination of mucosal epithelium deep into the wall** of the gallbladder. They represent herniations or diverticula resulting from increased intraluminal pressure.

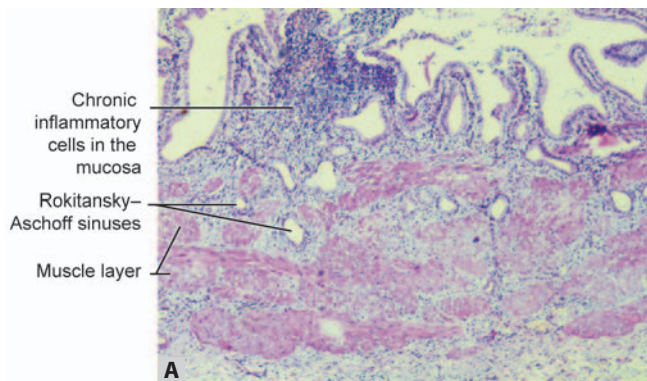
Rokitansky–Aschoff sinuses: Irregular, tubular structures formed due to invagination of gallbladder mucosal epithelium deep into the wall.

Morphological Variants

- **Cholecystitis glandularis:** In this variant **mucosal folds fuse together** and form crypts of epithelium buried in the gallbladder wall.



Figs 19.28A and B: Gross appearance of chronic cholecystitis: (A) Specimen; (B) Diagrammatic



Figs 19.29A and B: Microscopic appearance of chronic cholecystitis. Outpouching of the mucosa through the wall forms Rokitsky-Aschoff sinus. Mucosa is infiltrated by inflammatory cells: (A) Photomicrograph; (B) Diagrammatic

- **Porcelain gallbladder:** It is characterized by **extensive dystrophic calcification** within the gallbladder wall (predominantly in the muscle and lamina propria).
- **Acute on chronic cholecystitis:** Superimposition of acute inflammation on chronic inflammation indicates acute on chronic cholecystitis.
- **Xanthogranulomatous cholecystitis:** It is characterized by **marked thickening of wall** and **diffuse or nodular collections of macrophages containing neutral fat and lipofuscin (ceroid) pigment**.

Clinical Features

- Usually present as **recurrent attacks** of either **steady or colicky epigastric or right upper quadrant pain**.
- Other features include **nausea, vomiting, and intolerance for fatty foods**.

Complications

1. **Bacterial superinfection** with cholangitis or sepsis.
2. **Gallbladder perforation and local abscess formation**.
3. **Gallbladder rupture with diffuse peritonitis**.
4. **Biliary enteric (cholecystenteric) fistula**, with passage of gallstones into adjacent organs, such as intestine. It may result in **gallstone-induced intestinal obstruction (ileus)**.
5. **Porcelain gallbladder** has increased risk of cancer.

CHOLELITHIASIS (GALLSTONES)

- Cholelithiasis is a **common disorder** and found in ~10 to 20% of adult populations in developed countries.
- **Majority (>80%) are “silent”**.

Classification of Gallstones

Q. Classify gallstones/cholelithiasis.

Depending on the Chemical Composition

Chemical composition of gallstones: Cholesterol, calcium bilirubinate and other calcium salts (carbonate, phosphate and palmitate) are basic constituents.

Most widely used classification is based on the relative amount of cholesterol within stones. There are two main types of gallstones:

Cholesterol Gallstones

- They contain **more than 90% of crystalline cholesterol monohydrate** + calcium bilirubinate + calcium carbonate. **Cholesterol stones composed of mainly cholesterol are radiolucent.**
 - **Mixed cholesterol gallstones:** They contain a **mixture of cholesterol (60–89%)** + calcium bilirubinate + calcium carbonate + mucin glycoprotein.

Pigment Gallstones

- They develop when there is **increased unconjugated bilirubin**. They are composed of **calcium bilirubinate (calcium salts of unconjugated bilirubin)** + calcium carbonate + **less than 20% of cholesterol**. Pigment stones are subclassified as **black and brown pigment stones**.
 - **Black pigment stones:** They are common in India (40%).
 - ♦ Generally occur in patients with older age, cirrhosis and chronic hemolytic states.

- ◆ Contains 10–90% calcium bilirubinate + other calcium salts (50–75%—such as phosphate and carbonate), mucin glycoprotein. They have very low cholesterol concentration.
- ◆ 50–75% are radiopaque because of calcium salts.
- **Brown pigment stones:** These are more common in Asia.
 - ◆ They form in the bile duct and are associated with bile stasis and infections in the gallbladder and biliary tree. They are rare in the gallbladder.
 - ◆ Composed of calcium bilirubinate + calcium palmitate (calcium salts of free fatty acids) + mucin glycoprotein + fatty acids and cholesterol less than 20%. Calcium phosphate and calcium carbonate are usually not present (in contrast they are present in black pigment stones).
 - ◆ Usually radiolucent.

Old classification is presented in Box 19.5.

BOX 19.5: Older classification of gallstones

Pure gallstone (10%)

- Cholesterol
- Bile pigment (bilirubin)
- Calcium carbonate

Mixed stone (80%): Mixture of cholesterol, bile pigment and calcium carbonate in varying proportion.

Combined stone (10%): Pure gallstone nucleus with mixed stone shell or mixed gallstone nucleus with pure gallstone shell.

Depending on the Location

- **Intrahepatic stones:** They are predominantly **brown pigment stones**.
- **Gallbladder stones:** These are **mainly cholesterol stones** and **few are black pigment stones**.
- **Choledocholithiasis (bile duct stones):** Composed mostly of **mixed cholesterol stones**.

Risk Factors for Cholesterol Stones (Box 19.6)

Q. Write short note on risk factors for cholesterol stones.

Cholesterol gallstones are uncommon in developing countries. They are formed probably due to combination of genetic susceptibility and environmental factors.

BOX 19.6: Risk factors for gallstones

Cholesterol Stones

1. **Advancing age**
2. **Female sex hormones:** Female, pregnancy, parity and oral contraceptives
3. **Environmental factors:** Drugs (octreotide, ceftriaxone, clofibrate therapy), obesity and metabolic syndrome, rapid weight reduction, diet high in calories and cholesterol
4. **Acquired disorders:** Gallbladder stasis
5. **Genetic predisposition**
6. **Hereditary factors:** Familial predisposition, inborn disorders of bile acid metabolism
7. **Metabolic abnormalities:** Diabetes, genetic hyperlipoproteinemias and PBC

Pigment Stones

1. **Demography:** More common in Asians and in rural region more than urban
2. **Chronic hemolysis**
3. **Chronic biliary tract infections**
4. **Gastrointestinal diseases:** Ileal disease (e.g. Crohn disease), ileal resection or bypass, cystic fibrosis with pancreatic insufficiency
5. **Alcoholic cirrhosis**
6. **Pernicious anemia**
7. **Advancing age**

5 Fs of cholesterol stone:

Fat

Fertile

Forty

Female

Familial.

1. **Advancing age:** It is one of the **major risk factor**. Prevalence of cholesterol gallstones increases with age until, at 60 years. As age advances increase in gallstone is associated with the metabolic syndrome and obesity.
2. **Female sex hormones:**
 - **Female gender:** Gallstone is **two times more** common in females than in males and higher prevalence is observed in premenopausal women and both may be due to effect of estrogen. **Estrogen** increases the secretion of cholesterol and decrease the secretion of bile acids by the liver → favors → lithogenic bile secretion by the liver.
 - **Pregnancy:** In the last trimester of pregnancy the **gallbladder empties more slowly** and causes **stasis** and increases the **precipitation of cholesterol**

crystals. Progesterone, which is the predominant hormone during pregnancy inhibits the discharge of bile from the gallbladder.

- **Oral contraceptives:** They increases both uptake and synthesis of cholesterol.
3. **Environmental factors**
 - **Drugs:** Clofibrate, used to lower blood cholesterol results in **excessive secretion of cholesterol in the bile**. Drugs, such as octreotide and ceftriaxone also predispose to gallstones.
 - **Obesity and rapid weight loss:** These are strongly associated with **increased biliary cholesterol secretion**. Obesity enhances cholesterol absorption, synthesis, and secretion. There is probably a linear relation between body weight and the risk of gallstones.
 - **Diet high in calories and cholesterol:** They are associated with increased risk.
 4. **Acquired disorders: Gallbladder stasis** (neurogenic or hormonal) → promote cholesterol and pigment gallstones.

5. **Genetic predisposition:** Prevalence of cholesterol stones is highest in North American Indians, Chilean Indians.

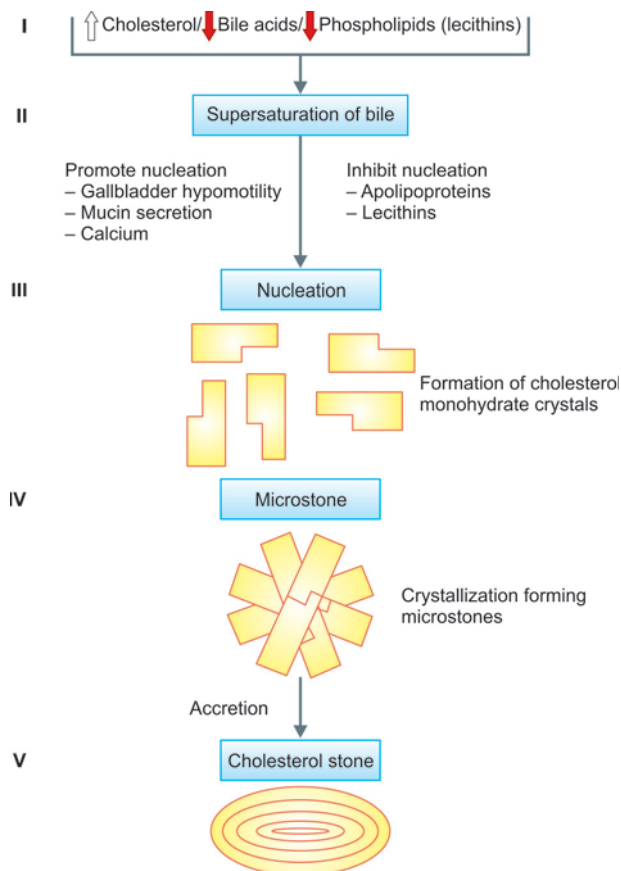
6. **Familial predisposition:** Gallstones are twice as common in first-degree relatives of patients with gallstones. *ABCG5* and *ABG2* genes participate in biliary cholesterol secretion and its variants increases the risk for the development of cholesterol gallstones.

7. **Metabolic abnormalities:** These associated with high blood cholesterol levels (e.g. diabetes, some genetic hyperlipoproteinemias, and PBC).

Pathogenesis of Cholesterol Stones (Fig. 19.30)

Q. Write short note on pathogenesis of cholesterol stones.

The cholesterol stone formation is **complex multifactorial process**. Cholesterol gallstone formation involves four simultaneous processes namely, 1. cholesterol supersaturation, 2. gallbladder hypomotility, 3. accelerated



Bile salts=Bile acids (cholic acid and chenodeoxycholic acid)+ taurine or glycine.

Bile acids: Water-soluble sterols and are the major catabolic products of cholesterol.

Bile acids: Act as detergents and solubilize water-insoluble lipids (secreted by hepatocytes) in the bile, and dietary lipids in the lumen of the gut.

Increased biliary cholesterol:

- Obesity
- Cholesterol-rich diet
- Clofibrate therapy.

Decreased bile acids:

- Primary biliary cirrhosis
- Mutation of CYP7A1 gene
- Oral contraceptive pills
- Impaired enterohepatic circulation (e.g. Crohn disease, ileal resection).

Decreased biliary lecithin: MDR-3 gene mutation.

Fig. 19.30: Pathogenesis of cholesterol stones

nucleation and crystallization and 4. growth to stone-sized aggregates.

Cholesterol Supersaturation

Major solutes of bile:

1. Bile acids (80%)
2. Phospholipids (includes lecithin and other phospholipids—16%)
3. Unesterified cholesterol (4.0%).

- **Cholesterol is secreted by the hepatocytes as vesicles (unilamellar or multilamellar) or micelles.**
- **Vesicles** are spherical structures consisting of **cholesterol surrounded by one or more layers of phospholipid bilayers** but **does not contain bile salts**.
 - **Unilamellar vesicle:** It consists of **cholesterol surrounded by one phospholipid bilayer**.
 - **Multilamellar vesicle:** It consists of **cholesterol surrounded by more than one phospholipid bilayer**.
- **Micellation:** It is the **process of conversion of vesicle into complex aggregates of water-soluble molecules** known as **mixed micelles** by the **detergent-like action of bile acids**.
 - **Mixed micelles** = bile acids + phospholipids + cholesterol, in which the lipophilic (fat soluble) portions of the three molecules aggregate in the center of the structure while the hydrophilic (water soluble) portions are oriented at the periphery, thus allowing solubility in bile.
- **Supersaturation:** It is a condition in which **cholesterol concentrations of bile exceed the solubilizing** (achieved by phospholipids and bile acids) **capacity of bile**. It is one of the important mechanisms in the formation of lithogenic (stone-forming) bile.

Primary bile acids: Includes cholic acid and chenodeoxycholic acid and are synthesized from cholesterol in the liver.

- **Causes of cholesterol supersaturation:** It may be due to **increased biliary cholesterol secretion** (most important cause), **decreased bile acid synthesis**, or **both**.
 - **Increased biliary cholesterol secretion:** It is observed in obesity, high-caloric diet and cholesterol-rich diets, certain drugs (e.g. clofibrate), pregnancy, oral contraceptives, rapid weight loss, and increased activity of HMG-CoA reductase (the rate limiting enzyme of hepatic cholesterol synthesis).
 - **Decreased bile salts:** e.g. Crohn disease.

Solubility of cholesterol in bile depends on the concentration of phospholipids and bile acids.

Gallbladder Hypomotility

Gallbladder hypomotility/stasis:

- Total parental nutrition (TPN)
- Prolonged fasting
- Pregnancy
- Massive burns
- Oral contraceptive pills
- Octreotide.
- Normal motility of the gallbladder empties and completely excretes its bile along with all supersaturated bile or crystals. Thus, motility does not allow stones to grow.
- Abnormalities in gallbladder emptying (decreased motility/hypomotility) → **causes gallbladder stasis** → **promote the aggregation of cholesterol crystals** → **formation of biliary sludge** (also known as microlithiasis).

Nucleation and Precipitation of Cholesterol Monohydrate Crystals

Nucleation

With supersaturation, cholesterol can no longer remain soluble.

Excess of cholesterol is carried as unilamellar cholesterol-rich vesicles → which fuse into large multilamellar vesicles → they nucleate to form solid microscopic cholesterol monohydrate crystals. The **nucleation** is the first and crucial step in gallstone formation and is **accelerated in lithogenic bile**.

Accelerated Nucleation

It may be due to either an excess of pronucleating factors or a deficiency of antinucleating factors.

- **Pronucleating factors:** **Mucin** and **certain non-mucin glycoproteins** and **biliary calcium** are important pronucleating factors.

Pronucleating factors:

- Mucin
- Non-Mucin glycoproteins
- Calcium
- Infection.

- **Hypersecretion of mucus:** Mucin glycoproteins are normally secreted continuously from the gallbladder. Hypersecretion of mucin **accelerates growth and precipitation of cholesterol monohydrate crystals**. Mucus traps the nucleated crystals and favors their aggregation into stones.

- **Calcium salts:** Calcium carbonate, calcium bilirubinate and calcium phosphate in the bile can serve as a nidus for cholesterol crystallization.
- **Anti-nucleating factors:** These include apolipoproteins A-1 and A-2, other glycoproteins and lecithins.

Anti-nucleating factors:

- Apolipoproteins A-1 and A-2
- Other glycoproteins
- Lecithins.

Growth to Stone-sized Aggregates

- **Biliary sludge:** It is defined as suspension of precipitates of cholesterol monohydrate crystals or calcium bilirubinate granules in bile. Before the appearance of stones, there is always the formation of a 'biliary sludge.' It implies supersaturation of bile with either cholesterol or calcium bilirubinate. Biliary sludge can resolve, persist, or progress. When it progresses to stones its crystals grow to form plates, in part because of impairment in gallbladder contractility.
- **Microlithiasis:** After nucleation, crystallization occurs, and cholesterol monohydrate crystals grow into microstones (microlithiasis/crystals in bile). Microlithiasis is identified by the presence of sludge by ultrasonography (US) or the presence of birefringent cholesterol crystals in bile.
- **Gallstone:** The microstones increase in size by gradual addition of more cholesterol and other constituents → form macroscopic gallstones.

Pathogenesis of Pigment Stones

Q. Write short note on risk factors/ pathogenesis of pigment stones.

Risk factors for pigment stones are listed in Box 19.6. Pigment stones are subclassified as **black and brown pigment stones**.

Black Pigment Stones

Black pigment stones associated with:

- Chronic extravascular hemolysis (e.g. hereditary spherocytosis, sickle cell anemia)
- Cirrhosis
- Mechanical prosthetic valves
- Gilbert syndrome
- Cystic fibrosis
- Ileal disease or resection.

- **Unconjugated bilirubin is insoluble in bile.** Under normal physiologic conditions, it is not secreted into bile and constitutes less than 1% of total bile pigment.
- **Increased production of unconjugated bilirubin** may precipitate as calcium bilirubinate probably around a nidus of mucinous glycoproteins to form black pigment stones.
- **Causes of increased unconjugated bilirubin in bile:** These include **chronic hemolysis** (e.g. β -thalassemia, hereditary spherocytosis, sickle cell disease), cirrhosis, severe ileal dysfunction (or bypass) and pancreatitis. In most patients, the predisposing cause is not known.

Brown Pigment Stones (Fig. 19.31)

Brown stones:

- Formed in bile duct
- Rare in gallbladder.

It can be found in the gallbladder or within the biliary tree.

Bile Stasis and Infection

Brown pigment stones develop secondary to:

- Bile stasis
- Biliary infection.

Brown pigment stones develop secondary to stasis and infection.

- **Stasis favors the bacterial infection** as well as accumulation of mucus.
- **Biliary infections**
 - **Bacterial infections of biliary tract by enteric bacteria** (predominantly *E. coli*) and with ascending cholangitis favor brown stone formation.
 - **Parasitic infestations:** Brown stones may be found in association with infestation by *Ascaris lumbricoides* or *Clonorchis sinensis*, and other helminthes, which may invade the biliary tract.
 - Dead bacteria and parasites may act as nuclei and accelerate the precipitation of calcium bilirubinate.

Actions of Bacterial Enzymes on Bile Constituents

In the bile, the bacterial enzymes have following actions:

- **Bacterial enzyme β -glucuronidase** → hydrolyzes conjugated bilirubin in the bile → to unconjugated, insoluble bilirubin.
- Bacterial **phospholipases hydrolyze phospholipids** and liberate free fatty acids (such as palmitic acid and stearic acid).

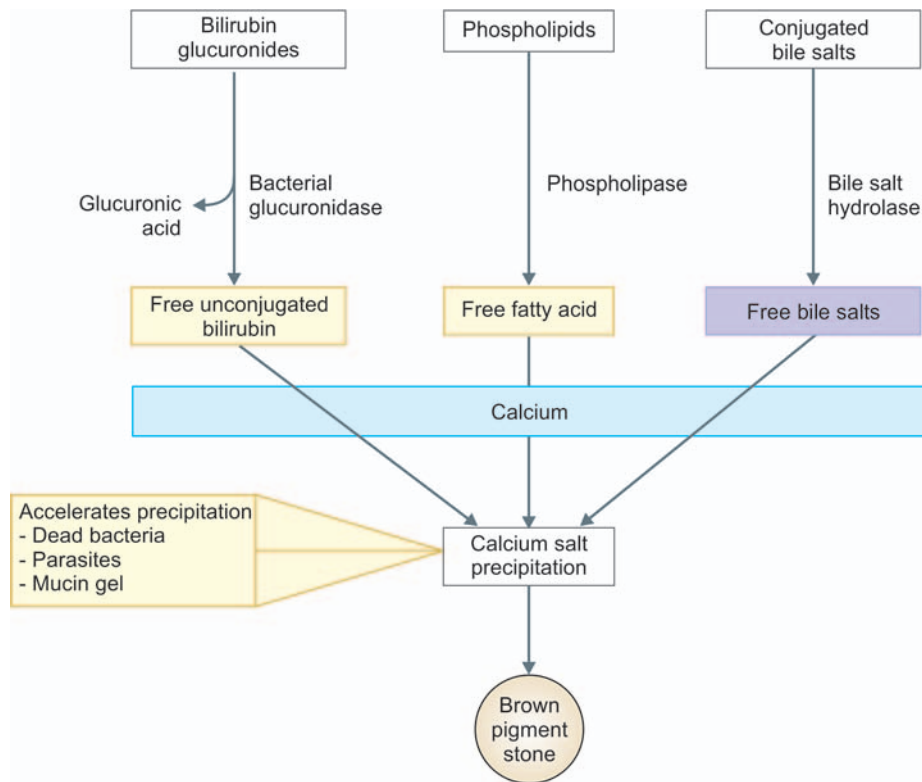


Fig. 19.31: Pathogenesis of brown pigment stones. The enteric bacteria produce β -glucuronidase, phospholipase A, and conjugated bile acid hydrolases. β -glucuronidase hydrolyze unconjugated bilirubin; phospholipase A liberates free fatty acids from phospholipids; and conjugated bile acid hydrolases produce unconjugated (free) bile acids. They form complex with calcium to produce insoluble calcium salts, thereby resulting in stone formation. Dead bacteria and parasites accelerate the precipitation of calcium bilirubinate. The mucin gel in the gallbladder can trap these complex precipitates and facilitate their growth into macroscopic stones

- Bacterial enzymes **hydrolyze bile salts** (glycine or taurine-conjugated) into **free (unconjugated) bile acids**.

Formation of Brown Stone

The **products** formed from above bacterial actions: 1. unconjugated insoluble bilirubin, 2. free fatty acids and 3. unconjugated bile acids. They **combine with calcium** to produce \rightarrow **water-insoluble calcium bilirubinate** \rightarrow forms brown stone.

MORPHOLOGY

Cholesterol Stones (Fig. 19.32A)

Cholesterol stones: Most common gallstone.

Cholesterol stones: Pure or mixed.

- Site: They occurs only in the gallbladder.
- Appearance: It **varies depending on the cholesterol content**; either purely cholesterol (100%—rare) or have cholesterol as the major chemical component.

Pure Cholesterol Stones

They account for 10% of gallstones.

Appearance: Single, pale yellow and round to ovoid.

- **Finely granular** and hard external surface.
- **Large** and measure 2–4 cm in diameter.
- **Cut surface:** It is **glistening** and shows **long, thin radiating cholesterol monohydrate crystals**.
- Radiolucent.

Mixed Gallstones

Stones with **lower cholesterol content** than the pure cholesterol stones are designated mixed. They are composed of **60–89% cholesterol** and **proportions of calcium carbonate, phosphates, and bilirubin**. They are **slightly more common** than pure cholesterol stones.

Appearance: Often multiple and gray-white to black.

- **Surfaces may be rounded or faceted**, because of tight apposition.
- **Smaller than pure cholesterol stones** and that range from 0.2 to 3.0 cm in diameter.

- **Cut surface:** Laminated with a dark core.
- **Stones composed largely of cholesterol** are radiolucent; but those with sufficient calcium carbonate are radiopaque (10–20%).

Pigment Gallstones

Black pigment stones are **formed in sterile gallbladder bile**, and **brown stones are formed in infected intrahepatic or extrahepatic ducts**.

Black Pigment Stones (Fig. 19.32B)

Black pigment stones: Seen with chronic extravascular hemolysis and consists mainly calcium bilirubinate.

Composed of either **pure calcium bilirubinate** or in **combination with a variety of other calcium salts** (hydroxyapatite and carbonate).

Appearance: Multiple with an **inverse relationship between size and number**.

- **Shiny, black or deep brown.**
- **Usually irregular, spiculated ("jacklike") molded** and may crumble touch.
- **Relatively small** and range from **0.2 to 1.5 cm in diameter**.
- **Arise in sterile gallbladder bile.**
- Because of calcium carbonates and phosphates, **approximately 50–75% are radiopaque**.

Brown Pigment Stones

Brown pigment stones: Sign of biliary tract infection.

Composed of **calcium salts of unconjugated bilirubin**, with **varying amounts of cholesterol and protein**.

- **Appearance:** Multiple and dull brown.
- **Laminated and soft** and may have a **soap-like or greasy consistency**. These stones are **easy to crush endoscopically**.
- Range from **0.2 to 1.5 cm in diameter**.
- **Usually radiolucent**.
- **Usually associated with infection of biliary tract and intrahepatic or extrahepatic ducts**.

Clinical Features

- Majority of patients with gallstones remain **asymptomatic** throughout their lives.
- Gallstones usually produce symptoms by causing inflammation or obstruction when they pass into the cystic duct or CBD.
- The characteristic symptom is **biliary colic** that is a constant and often long-lasting pain. Large gallstones usually cannot enter the cystic or common ducts to produce obstruction.

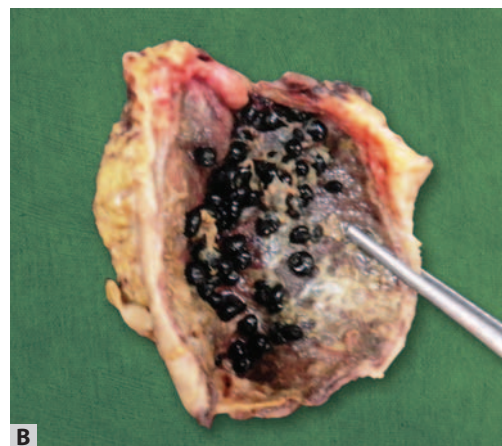
Complications

Q. Write short note on complications of gallstones.

In the Gallbladder

Common complications of gallstones:

1. Cholecystitis
 2. Biliary obstruction
 3. Carcinoma
 4. Acute pancreatitis.
- **Cholecystitis:** It can cause both **acute and chronic cholecystitis**. Rarely it may cause gangrenous or emphysematous cholecystitis.
 - **Empyema of the gallbladder:** It is characterized by distention of gallbladder with pus.
 - **Hydrops of the gallbladder:** Gallbladder distended with clear watery fluid.
 - **Mucocele:** Gallbladder distended by cloudy, mucoid material.
 - **Perforation of gallbladder.**
 - **Carcinoma:** Gallstones may **increase the risk of carcinoma** of the gallbladder.



Figs 19.32A and B: Gallbladder with stones: (A) Showing cholesterol and mixed stones; (B) Showing multiple black pigment stones

In the Bile Ducts

Hydrops of gallbladder: Due to obstruction of cystic duct.

- **Biliary obstruction** (obstructive cholestasis).
- **Acute cholangitis** (inflammation of the biliary tree).
- **Acute pancreatitis**.

In the Intestine

- **Biliary fistulas:** Fistula may develop **between biliary system and bowel or gallbladder and skin**.
- **Gallstone ileus or Bouveret's syndrome:** Rarely a large gallstone may erode directly into an adjacent loop of small intestine and produce intestinal obstruction ("gallstone ileus" or Bouveret's syndrome).

CARCINOMA OF THE GALLBLADDER

- Carcinoma of the gallbladder **rare**; however, it is the most common malignancy of the extrahepatic biliary tract.
- It is slightly **more common in females** and occurs most frequently during seventh decade of life.
- It is common in parts of northern India.

Etiology

Risk Factor

Risk factors for carcinoma of gallbladder:

- Gallstones
- Porcelain gallbladder.
- **Gallstones:** They predispose to **carcinoma by causing chronic irritation, chronic inflammation and epithelial damage**.
- **Genetic factors:** Prevalence of the gallbladder cancer in American Indians, Hispanic Americans and parts of north India probably reflect the role of genetic factors.
- **Carcinogen:** **Present in the bile acids** are believed to play a role.
- **Calcification of the gallbladder.**
- **Gastrointestinal disorders:** Ulcerative colitis and polyposis syndromes also have an association with gallbladder adenocarcinoma.

Molecular Genetics

- Carcinoma of the gallbladder develops through accumulation of multiple genetic alterations, involving

oncogenes (*KRAS* mutation), tumor suppressor genes (*p53* mutations), and DNA repair genes.

- Microsatellite instability is present in about 10% of cases.

MORPHOLOGY

Gross

- Sites: **Most arise in the fundus and neck** (60%), followed by the body (30%).
- **Usually show calculi** and marked fibrosis of the wall.
- **Two patterns of growth:**
- **Diffusely infiltrating (70%):** It usually appear as a **poorly defined area of diffuse thickening of the gallbladder wall** (due to **prominent desmoplasia**) and similar to linitis plastica of stomach. These tumors are **scirrhous, gray-white** and have a very firm consistency. Deep ulceration can cause fistula formation to adjacent viscera.
- **Exophytic/polypoid (30%):** It grows into the lumen as an **irregular, cauliflower mass** and also invade the underlying wall of the gallbladder. The luminal portion of the tumor mass may show areas of necrosis, hemorrhage and ulceration.

Microscopy

Carcinoma of gallbladder: Adenocarcinoma.

- Most gallbladder cancers are **adenocarcinomas** showing varying degrees of differentiation.
- The well-differentiated carcinomas consist of well-formed glands lined by a layer of cuboidal cells and often embedded in a desmoplastic stroma.
- Others tumors may be infiltrative and poorly differentiated to undifferentiated.

Spread

Carcinoma of gallbladder: Most common symptom is biliary colic.

- **Direct spread:** Tumor may spread to liver, peritoneal surfaces, cystic duct and adjacent bile ducts. Biliary-enteric fistulas may develop when tumor penetrates into intestine.
- **Lymphatic spread:** It may spread to perihilar lymphatics and portal-hepatic lymph nodes and in later stages, mediastinal and supraclavicular lymph nodes.
- **Blood spread:** **Liver** (both local spread and hematogenous spread) and **lungs**.

Clinical Features

- **Right upper quadrant abdominal pain, anorexia and nausea and vomiting.**
- Laboratory investigation show **elevated alkaline phosphatase level**.

Pancreatic Disorders

ACUTE PANCREATITIS

Pancreatitis: Inflammation of exocrine pancreas may be acute or chronic.

- Inflammatory disease of the exocrine pancreas may be classified as (1) acute pancreatitis or (2) chronic pancreatitis.
- Acute pancreatitis is **acute inflammation of the exocrine pancreas** due to injury to the parenchyma of the pancreas.
- Acute pancreatitis is relatively common.

Acute pancreatitis: Inflammation and reversible damage to parenchyma.

Etiology

Q. Discuss the etiology and pathogenesis of acute pancreatitis.

Various causes of acute pancreatitis are presented in Box 20.1. **Alcoholism and gallstones account for about 80% of the cases.**

1. **Alcohol:** It causes acute pancreatitis usually in individuals who consume large quantities of alcohol. A **polymorphism in the detoxifying enzyme uridine 5-diphosphate (UDP) glucuronyl transferase increases the risk for alcoholic induced pancreatitis.** The male-to-female ratio is 6:1.
2. **Gallstones:** It is responsible for acute pancreatitis **in about 30–60% of cases.** The frequency of acute pancreatitis is inversely proportional to the size of gallstones. The male-to-female ratio is 1: 3.

Acute pancreatitis:

Gallstones most common cause

Alcohol abuse 2nd most common cause.

BOX 20.1: Causes of acute pancreatitis

Metabolic

- **Alcoholism**
- Hyperlipoproteinemia
- Hyperparathyroidism
- Hypercalcemia.

Mechanical

- **Gallstones**
- Trauma blunt abdominal trauma
- Iatrogenic injury: During surgery (e.g. endoscopic procedures with dye injection)
- Periapillary neoplasms (such as pancreatic cancer)
- Parasites (*Ascaris lumbricoides* and *Clonorchis sinensis* organisms).

Drugs and toxins

- Drugs: Furosemide, azathioprine
- Toxins: Insecticides, methanol, organophosphates.

Genetic

- Mutation in cationic trypsinogen (*PRSS1*) and trypsin inhibitor (*SPINK1*) gene.

Vascular

- Shock
- Atheroembolism
- Thrombosis and embolism
- Vasculitis.

Infectious

- Viral infections: Mumps, coxsackie virus.

Idiopathic (~10–20%)

3. **Genetic factors:** No cause is identified in about 10–20% of patients and are termed idiopathic. Some of these may have a genetic basis. Three susceptibility genes have been identified:

Genetic factors in acute pancreatitis: Mutations in genes: *PRSS 1*, *SPINK 1*, *CFTR*.

MORPHOLOGY**Q. Write short note on morphology of acute pancreatitis.**

The changes depend on the duration and severity of the process. Morphologically, acute pancreatitis classified into 3 types.

Morphological types of acute pancreatitis:

1. Interstitial
2. Necrotizing
3. Hemorrhagic.

Acute Interstitial or Edematous Pancreatitis

- It is a **mild and reversible** process.
- **Microscopy:** It shows **interstitial edema** and **mild infiltration** by **polymorphonuclear leukocytes**. **Necrosis or hemorrhage is not seen.**

Acute Necrotizing Pancreatitis**Gross**

- **Pancreas:**
 - It is **enlarged** and **swollen**, and shows **red-black areas of hemorrhage** with foci of **yellow-white, chalky fat necrosis** (refer page 20 and Fig. 1.21). Fat necrosis is due to the action of lipase on triglycerides, which release fatty acids from the fat cells.
 - The **fatty acids combine with calcium to form insoluble salts** (calcium soaps). This process of soap formation is known as **saponification**. The calcification **may reduce the level of blood calcium**, sometimes to such an extent of causing neuromuscular irritability.
- **Extrapaneatritic lesions:**
 - **Foci of fat necrosis** (refer Fig. 1.21): It may also be seen in **extrapancreatic fat** (omentum and the mesentery of the bowel) and outside the abdominal cavity (subcutaneous fat).
 - **Fluid:** The **peripancreatic tissue or peritoneal cavity** may contain a **serous, slightly turbid, brown-tinged fluid**. This fluid may show **necrotic fat globules**, which are produced by the action of enzymes on adipose tissue. The necrotic tissue appears **“putty-like,”** and is similar to **“canned dog food”**.

Microscopy (Fig. 20.1)

Microscopy of acute pancreatitis:

Acute inflammation

Edema

Enzymatic fat necrosis

Hemorrhage.

- **Edema of interstitial tissue:** It due to leakage of fluid from the microvasculature.
- **Acute inflammation:** Seen in the interstitial tissue.
- **Enzymatic fat necrosis** (Fig. 1.21): It appears as **granular blue** with **ghost outlines of the necrotic cells**.
- **Destruction of parenchyma of the pancreas:** Acinar and ductal tissues as well as the islets of Langerhans are necrotic due to **proteolytic digestion**.

- **Destruction of blood vessels:** The **activated elastase** destroys blood vessel and leads to **interstitial hemorrhage**.

Acute Hemorrhagic Pancreatitis

- It usually develops in **middle age** (peak incidence at 60 years) and associated with **high morbidity and mortality**.
- **Gross (Fig. 20.2):** It shows **extensive necrosis, hemorrhage** and **fat necrosis** within the **pancreatic parenchyma**. In severe cases, marked hemorrhage may convert the pancreas into a large retroperitoneal hematoma.

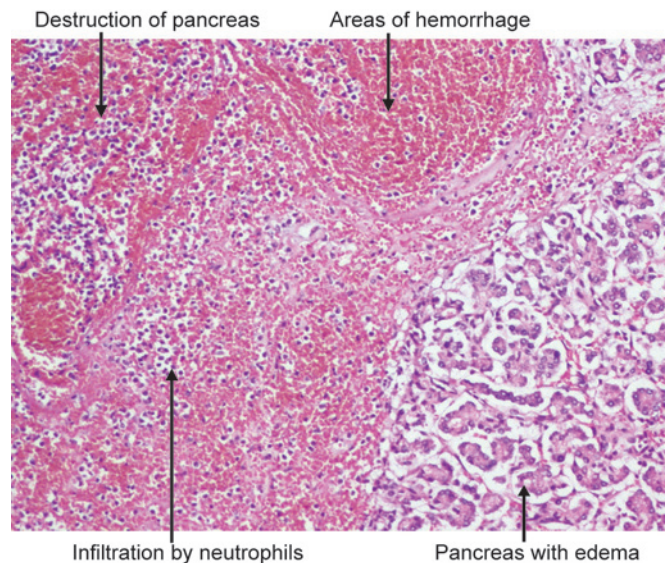


Fig. 20.1: Microscopic appearance of acute hemorrhagic pancreatitis. Histology shows numerous neutrophils, hemorrhage and destruction of parenchyma (left side) and pancreas with edema (right side)

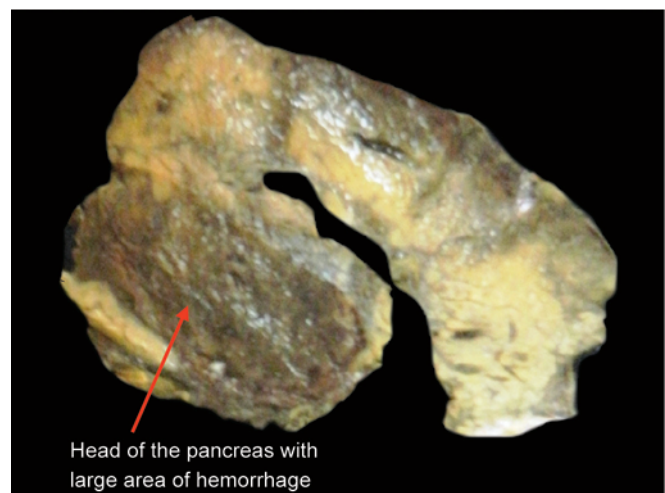


Fig. 20.2: Acute hemorrhagic pancreatitis. Longitudinal section of the pancreas shows dark areas of hemorrhage in the head of the pancreas (left)

- **Mutation in cationic trypsinogen (*PRSS1*) gene:** It is the **most common genetic defect**, which results in resistance to trypsin hydrolysis.
 - **Mutation in the pancreatic trypsin inhibitor gene** (serine protease inhibitor, Kazal type 1; *SPINK1*): *SPINK1* gene is essential for the inactivation of trypsin and for the prevention of the autodigestion of the pancreas by activated trypsin. Mutation of this gene **causes inappropriate activation of trypsin**, which in turn can **activate other digestive proenzymes** resulting in pancreatitis.
 - **Mutations in the cystic fibrosis transmembrane regulator (*CFTR*) gene.**
4. **Drugs:** They cause pancreatitis **either by a hypersensitivity reaction** or by the generation of **toxic metabolite**.

Pathogenesis (Fig. 20.3)

Safety mechanism in pancreas to prevent autoactivation of trypsin: Pancreatic secretory trypsin inhibitor (PSTI) binds with the active trypsin and inhibits its activity.

The exact mechanisms by which these causes trigger inflammation of the pancreas are not completely known.

Autodigestion of the Pancreatic Substance

According to this theory, pancreatitis **develops due to premature (inappropriate) activation of proteolytic pancreatic enzymes** → leads to a **process of autodigestion**.

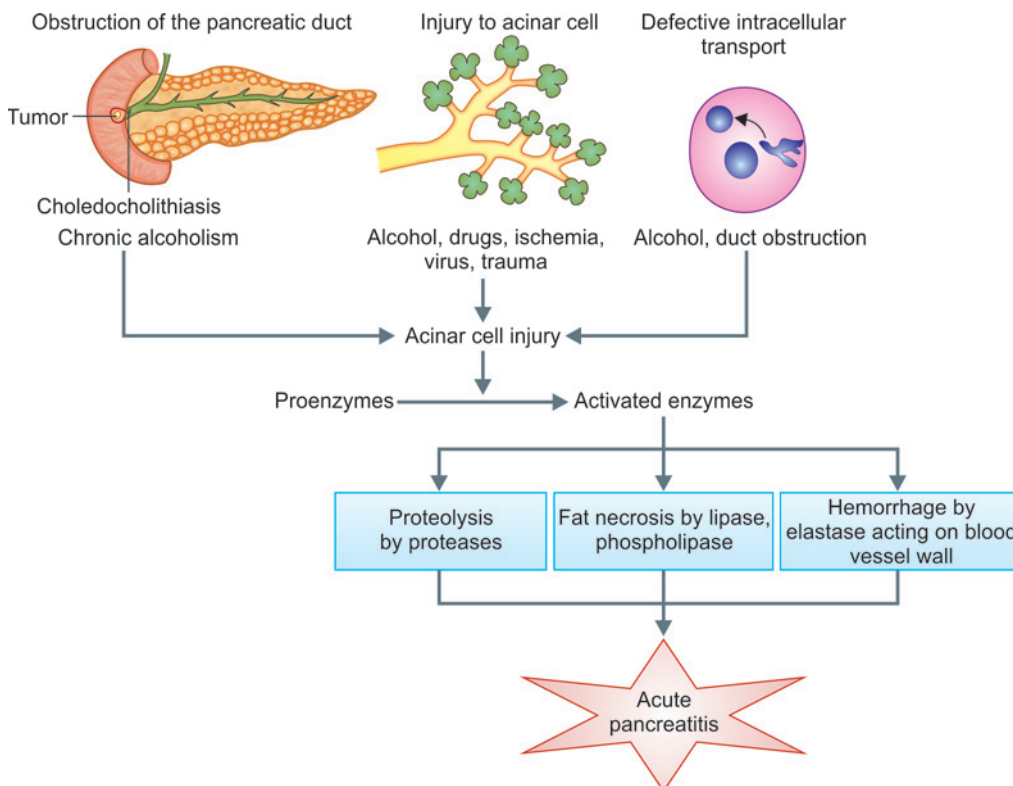
Activation of Pancreatic Enzymes

Pancreatic enzymes are synthesized in an inactive proenzyme form (e.g. trypsinogen, chymotrypsinogen, proelastase, and lipolytic enzymes, such as phospholipase A₂) and become active when converted into enzyme form. **Premature activation** (before their secretion from the acinar cells) of **pancreatic enzymes** is responsible for acute pancreatitis.

- **Activation of trypsin:** One of the pancreatic **proenzyme trypsinogen is prematurely activated to trypsin** and it is an **important triggering event** in the pathogenesis of **acute pancreatitis**.

Acute pancreatitis: Important triggering event is prematurely activation of pancreatic proenzyme trypsinogen to trypsin.

- **Factors activating trypsin**, e.g. endotoxins, exotoxins, viral infections, ischemia, anoxia. Trypsin also can be spontaneous activated.



Mechanism of activation of pancreatic enzymes in acute pancreatitis:

1. Pancreatic duct obstruction
2. Primary acinar injury
3. Defective intracellular transport of proenzymes.

Fig. 20.3: Pathogenesis of acute pancreatitis

- **Actions of trypsin:** Trypsin can **convert many proenzymes into active forms** [e.g. proelastase to → elastase (damages the elastic fibers of blood vessels) and phospholipase to → phospholipase degrades fat cells].
- **Activation of kinin, clotting and complement system:** Trypsin acts on kinin system and converts prekallikrein to → kallikrein → activate Hageman factor (factor XII), the clotting and complement systems.

Mechanism of Activation of Pancreatic Enzymes

The exact mechanisms of activation of pancreatic enzymes are not well-understood. However, the fundamental mechanism that transforms the initial injury into pancreatitis appears to be **intracellular activation of digestive proenzymes into active enzymes in the pancreatic acinar cells**. This can involve **three possible pathways**:

1. **Pancreatic duct obstruction:** According to this theory, partial or total obstruction of the pancreatic duct alone can cause pancreatitis.
 - Any **pancreatic duct obstruction** (e.g. gallstones, biliary sludge, tumor) can **raise intraductal pressure** → exacerbate back-diffusion across the ducts → lead to the **rupture of ductules and acini** → **liberates enzyme-rich fluid** in the interstitium and **inappropriate activation of digestive proenzymes** → causes injury → initiate local inflammation and interstitial edema. Edema may decrease the local blood flow and cause further ischemic injury to acinar cells.
2. **Primary acinar cell injury:** Any injury to the acinar cell can trigger acute pancreatitis.
 - Direct acinar cell injury may be caused by certain **viruses (e.g. mumps), drugs, alcohol and direct trauma** to the pancreas. Injury may also follow ischemia or shock.
 - Cellular injury → **initiates the inflammatory process** → leads to pancreatic edema, hemorrhage → necrosis.
3. **Defective intracellular transport of proenzymes within acinar cells:** It is responsible for pancreatitis **due to metabolic injury, alcohol or duct obstruction**.
 - In pancreatic acinar cells, normally digestive enzymes and lysosomal hydrolases are transported in separate pathways.
 - **Fusion of lysosome and digestive enzymes within large vacuoles** → may activate the pancreatic proenzymes → cause acute intracellular injury.

Mechanism of Alcohol-induced Pancreatitis

Mechanism of alcohol-induced acute pancreatitis:

1. **Small ductules obstruction**
 2. **Abnormal sphincter of Oddi spasm**
 3. **Direct toxic effect**
 4. **Increased proteases.**
1. **Obstruction of small ductules by proteinaceous plugs:** Chronic alcohol ingestion results in the **secretion of protein-rich pancreatic fluid** → form **inspissated protein plugs** → obstruct small pancreatic ducts. They lead to pancreatitis similar to duct obstruction described above.
 2. **Abnormal sphincter of Oddi spasm:** Alcohol increases pancreatic exocrine secretion and causes **contraction of the sphincter of Oddi** (the muscle at the ampulla of Vater) → pancreatitis.
 3. **Direct toxic effects on acinar cells:** This may be caused by **alcohol and its metabolic byproducts**.
 4. **Increased amounts of proteases in pancreatic secretions:** Found in alcoholic patients.

Clinical Features

Acute pancreatitis: May present as acute abdominal medical emergency.

- **Abdominal pain:** It is constant and intense. It is **referred to the upper back** and its severity varies from mild to severe.
- **Other symptoms:** These include anorexia, nausea, and vomiting.

Laboratory Findings

- **Leukocytosis:** It is usually found in moderate to severe acute pancreatitis.
- **Serum amylase:** It is marked elevated during the **first 24 hours**.
- **Serum lipase:** It is raised **within 72–96 hours** and follows elevated amylase.
- **Glycosuria:** It occurs in 10% of cases.
- **Hypocalcemia:** It is **due to precipitation** of calcium soaps in necrotic fat.
- **Direct visualization of the pancreas:** By radiography.

Acute pancreatitis: C-reactive protein level >130 mg/mL indicates severe pancreatitis.

Acute pancreatitis: A three-fold or higher elevation of amylase and lipase levels confirm the diagnosis.

Acute pancreatitis: Lipase is a more specific/diagnostic marker and lasts longer. Amylase can be elevated in other conditions (e.g. peptic ulcer disease, mesenteric ischemia, salpingitis).

Acute pancreatitis: Persistent of hypocalcemia is a poor prognostic sign.

Complications of Acute Pancreatitis (Table 20.1)

TABLE 20.1: Complications of acute pancreatitis

Q. Write short note on complications of acute pancreatitis.

Local complications	Systemic complications
<ul style="list-style-type: none"> • Sterile pancreatic abscess • Pancreatic pseudocyst • Infection by gram-negative organisms from the alimentary tract 	<ul style="list-style-type: none"> • Shock with acute renal tubular necrosis during the first week • Acute respiratory distress syndrome • Acute renal failure • Hemolysis • Disseminated intravascular coagulation (DIC) • Diffuse fat necrosis

CHRONIC PANCREATITIS

Chronic pancreatitis: Irreversible damage to the parenchyma and scar formation.

Definition: Chronic pancreatitis is defined as **chronic inflammation** of the pancreas characterized by the presence of **permanent and progressive morphologic or functional damage to the pancreas**.

Pancreas shows **irreversible damage of exocrine parenchyma, and fibrosis**. In the **late stages**, there may be **destruction of endocrine parenchyma**.

Etiology

Chronic pancreatitis: Alcohol abuse is the most common cause.

- **Alcohol abuse:** **Chronic alcohol consumption** is the **most common (about 70%) cause** and usually develops in middle-aged males.
- **Less common causes:**
 - **Obstruction of the pancreatic duct:** By pseudocysts, calculi, trauma, or carcinoma.

- **Hyperparathyroidism:** May develop as a result of the hypercalcemia.
- **CFTR gene mutations:** **Cystic fibrosis** is caused by inherited mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. This **decreases bicarbonate secretion by pancreatic ductal cells** and **promote protein plugging** and the development of chronic pancreatitis.
- **Tropical pancreatitis:** It is seen in Africa and Asia and few have a genetic basis.
- **Hereditary pancreatitis.**
- **Autoimmune pancreatitis.**
- **Idiopathic:** It constitutes about 20% of cases.

Pathogenesis (Fig. 20.4)

Q. Discuss the pathogenesis of chronic pancreatitis.

The pathogenesis of chronic pancreatitis is not clear. **Repeated attacks of acute pancreatitis may lead to chronic pancreatitis**. **Four theories** have been suggested and it is unlikely that all cases of chronic pancreatitis can be explained by a single theory.

Pathogenesis of chronic pancreatitis

Four theories include:

1. Obstruction
2. Necrosis-fibrosis
3. Toxic-metabolic
4. Oxidative stress.

Obstruction Theory

Some etiological agents causes **increase protein concentrations** → precipitation of protein → form **ductal plugs** → calcification of ductal plugs to form calculi → further **obstruction of the pancreatic ducts**. They are found in **alcoholic induced chronic pancreatitis**.

Necrosis-fibrosis Theory

According to this hypothesis, the inflammation and scarring resulting from **bouts of acute pancreatitis** → cause **obstruction and stasis** within the ducts → form stones in the duct.

Toxic-metabolic Theory

This theory proposes that toxins such as alcohol and its metabolites can cause **direct toxic damage to acinar cells** → produces necrosis of cells → eventually fibrosis.

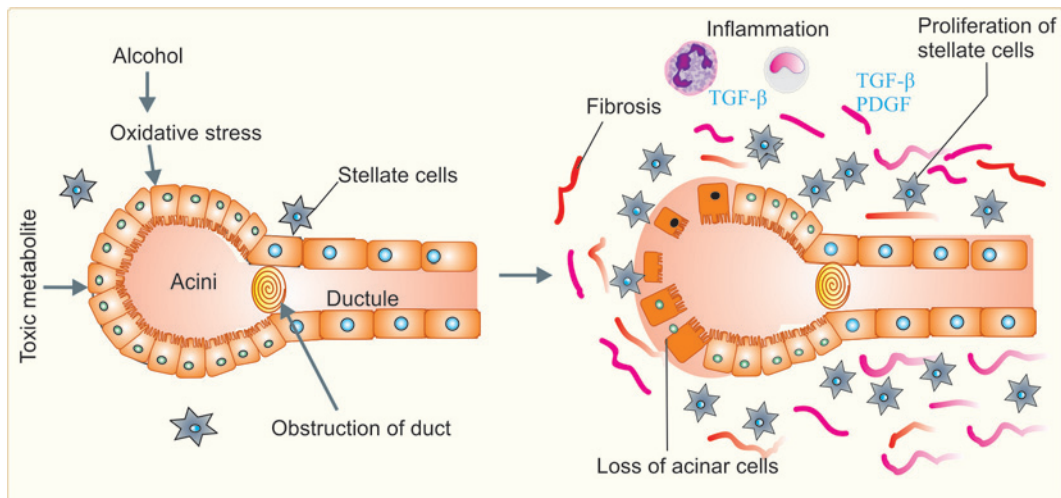


Fig. 20.4: Pathogenesis of chronic pancreatitis. Repeated episodes of acinar cell injury produces profibrogenic cytokines such as transforming growth factor β (TGF- β) and platelet-derived growth factor (PDGF), resulting in the proliferation of stellate cells (myofibroblasts) and produces fibrosis

Oxidative Stress Theory

- The pancreas is exposed to “oxidative stress” either through the systemic circulation or through reflux of bile into the pancreatic duct.
- **Oxidative stress generates free radicals** in acinar cells with following consequences:
 - Leads to membrane lipid oxidation and acinar cell necrosis.
 - Recurrent inflammation, tissue damage and fibrosis, and the fusion of lysosomes and proenzymes.
 - Alcohol abuse is one of the etiological factors, which induces oxidative stress.

Profibrogenic Chemokines

- Two profibrogenic chemokines namely **transforming growth factor β (TGF- β)** and **platelet-derived growth factor (PDGF)** have been found in chronic pancreatitis.
- They cause **activation and proliferation of peri-acinar myofibroblasts** (pancreatic stellate cells), which **deposit collagen** and ultimately lead to **fibrosis**.

MORPHOLOGY

Chronic pancreatitis:

1. Non-obstructive
2. Obstructive.

Gross

Chronic pancreatitis can be subdivided into two major forms:

Non-obstructive chronic pancreatitis

It is by far the **most common form of chronic pancreatitis** (~95%) and about 80% of the patients are alcoholics.

- Pancreas is **nodular, hard** and may be either enlarged or atrophic.
- **Cut section** may show **dilated ducts** and **calcified concretions**.
- **When the calcification is extensive**, it is termed as **chronic calcifying pancreatitis**.

Obstructive chronic pancreatitis

It is the result of narrowing or occlusion of the pancreatic ducts. It is most **commonly due to carcinoma and stones** in the ductal system.

Microscopy

Chronic pancreatitis is characterized by:

1. **Parenchymal fibrosis:** The pancreas shows large irregular areas of fibrosis (periductal, intralobular, and interlobular). It is a major feature.

Clinical Features

Steatorrhea: Seen in chronic pancreatitis and not in acute pancreatitis.

- **Repeated attacks of abdominal pain** or persistent abdominal and back pain.
- **Silent until severe functional damage** develops:

Complications of chronic pancreatitis
 Pancreatic pseudocysts
 Malabsorption, steatorrhea
 Secondary diabetes
 Risk of developing pancreatic cancer.

2. **Atrophy of acini:** There is reduction in the number and size of the acini. The islets of Langerhans are relatively resistant to chronic pancreatitis in comparison with the pancreatic acini. However, as the disease progresses they may be reduced or disappear.
3. **Dilation of the pancreatic ducts:** The interlobular and intralobular pancreatic ducts are frequently dilated and their lumen show protein plugs.
4. **Alterations in the duct epithelium:** It shows atrophy/hyperplasia/squamous metaplasia.
5. **Chronic inflammatory infiltrate:** Fibrotic areas show infiltration by lymphocytes, plasma cells, and macrophages.

Chronic pancreatitis:

1. Parenchymal fibrosis
2. Atrophy of acini
3. Dilatation of pancreatic ducts
4. Atrophy/hyperplasia/squamous metaplasia of duct epithelium
5. Chronic inflammation.

- **Pancreatic exocrine insufficiency:** Characterized by weight loss and hypoalbuminemic edema due to chronic malabsorption.
- **Pancreatic endocrine insufficiency:** Diabetes mellitus may develop.

Prognosis: Long-term outlook is poor.

PSEUDOCYST OF PANCREAS (FIG. 20.5)

Q. Write short note on pancreatic pseudocyst.

- Pseudocysts constitute about 75% of cysts in the pancreas.
- Pseudocyst is **not true cyst** and **does not have any epithelial lining**. It is intimately associated with the pancreatic tissues.
- **Causes:** Complication of acute and chronic pancreatitis, and traumatic injury to the pancreas.

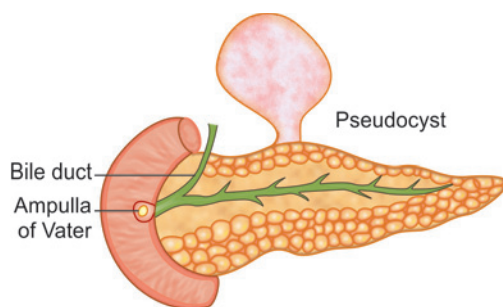


Fig. 20.5: Pseudocyst of pancreas

MORPHOLOGY

Gross

- Number: It is usually **single**.
- Size: It ranges from 2 to 30 cm in diameter.
- Sites (Fig. 20.5): May be seen **within the substance of the pancreas**, or **more commonly in the lesser omental sac** or in the **retroperitoneum** between the stomach and transverse colon or between the stomach and liver.
- Lumen: It contains **necrotic-hemorrhagic material rich in pancreatic enzymes** (amylase).

Microscopy

The wall of the pseudocysts shows non-epithelial-lined fibrous connective tissue and necrotic pancreatic debris.

Complications

Majority of pseudocysts resolve spontaneously.

- Pseudocyst may **enlarge and compress** or obstruct the duodenum or even perforate into **adjacent structures**.
- May be **secondarily infected** → form an **abscess**.
- Rarely may **rupture** → **chemical or septic peritonitis** or both.

PANCREATIC CARCINOMA

Infiltrating ductal adenocarcinoma of the exocrine pancreas (commonly known as “pancreatic cancer”) is the most frequent neoplasm of the pancreas (about 85% of all neoplasms).

Precursors to Pancreatic Cancer (Pancreatic Intraepithelial Neoplasias)

Pancreatic cancer: Probably arises from precursor lesions known as PanINs.

- Similar to colorectal cancer, there is a progression in the pancreas from non-neoplastic epithelium to well-defined noninvasive lesions in small ducts and ductules to invasive carcinoma.
- The precursor lesions are called “pancreatic intraepithelial neoplasias” (PanINs).

Carcinoma of pancreas:

More than 85% are ductal adenocarcinomas.

Molecular Carcinogenesis (Fig. 20.6)

1. **Telomere shortening:** The epithelial cells in PanINs show critical shortening of telomere length, which predisposes

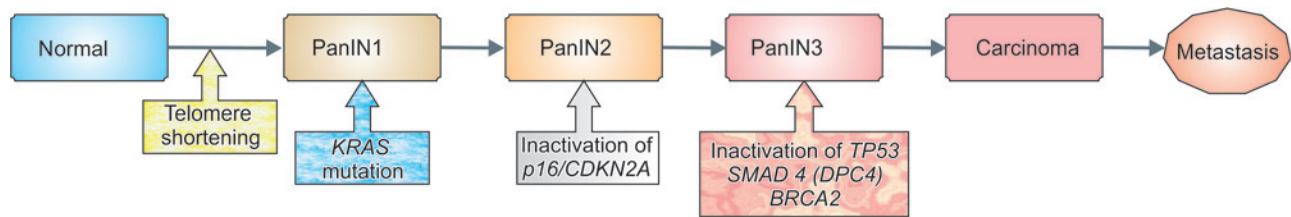


Fig. 20.6: Genetic progression of pancreatic carcinogenesis. The progression from normal epithelium to low-grade pancreatic intraepithelial neoplasia (PanIN) (PanIN1 and PanIN2), to high-grade PanIN3, to invasive carcinoma (left to right) is associated with the accumulation of specific genetic alterations. Telomere shortening and mutations of the oncogene *KRAS* occur at early stages. It is followed by inactivation of the *p16* (tumor suppressor gene) at intermediate stages, and the inactivation of the *TP53*, *SMAD4* (*DPC4*), and *BRCA2* tumor suppressor genes occur at late stages

these lesions to accumulate progressive chromosomal abnormalities and to develop invasive carcinoma.

2. Oncogene:

Adenocarcinoma of pancreas: Most common gene mutation (oncogene activated by point mutation) is *KRAS*.

- **Mutation of *KRAS*:** It is an early genetic alteration, which in turn activates several intracellular signal transduction pathways and the transcription factors Fos and Jun.

3. Tumor suppressor gene:

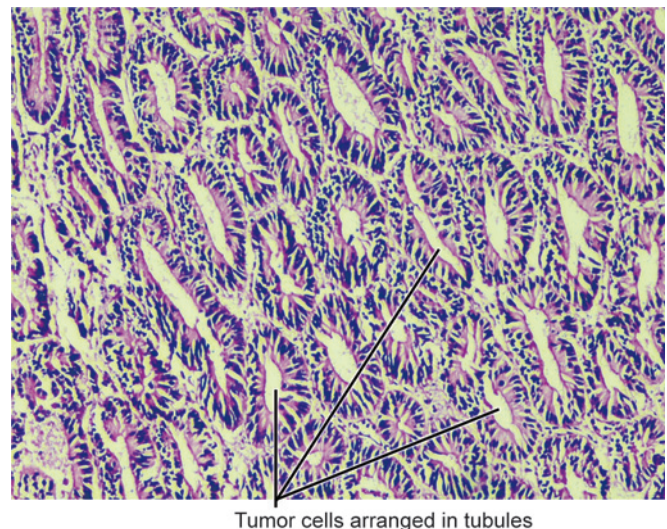
- **Inactivating mutations of *p16/CDKN2A*:** It occurs in 90% of the cases. The p16 protein (product of *p16*) plays an important role in the control of the cell cycle, and its inactivation results in loss of cell cycle checkpoint.

KRAS gene is located on chromosome 12p.

- **Mutational inactivation of *SMAD4*:** It occurs in about 55% of pancreatic cancers. *SMAD4* is only rarely inactivated in other cancers.
- **Mutations of *TP53*:** It is found in about 75% of pancreatic cancers.

Etiology, and Pathogenesis

- **Age:** It is usually found in the elderly patients and about 80% of cases occur between the ages of 60 and 80 years.
- **Risk factors:**
 - Cigarette smoking and alcohol use.
 - Consumption of a diet rich in fats.
 - Chronic pancreatitis.
 - Diabetes mellitus.
 - Inherited genetic defects:
 - ◆ *BRCA2* mutations
 - ◆ Mutations in *CDKN2A* (*p16*)
 - ◆ Mutation in the *PALLD* gene.



Tumor cells arranged in tubules

Fig. 20.7: Pancreatic carcinoma shows tumor cells forming abortive tubular structures

MORPHOLOGY

Pancreatic cancer

1. Highly invasive
2. Desmoplastic response.

- **Location:** About 60% in the head, 15% in the body, and 5% in the tail; in about 20% of cancers diffusely involve the entire pancreas.
- **Gross:** Pancreatic carcinomas are usually appear as hard, stellate, gray-white, poorly delineated and firm masses.
- **Microscopy:** Pancreatic ductal adenocarcinoma are graded microscopically into well-differentiated, moderately differentiated, and poorly differentiated. The tumor cells forms abortive tubular structures (Fig. 20.7) or cell clusters and show infiltration. The malignant glands are poorly formed and are usually lined by pleomorphic cuboidal-to-columnar epithelial cells.

Spread

Perineural invasion:

1. Pancreatic cancer
2. Prostatic cancer
3. Adenoid cystic carcinoma
4. Carcinoma of gallbladder
5. Cholangiocarcinoma.

- **Local spread:** Pancreatic cancers usually show **perineural invasion**. They grow along nerves and invade into the retroperitoneum. They can directly spread into peripancreatic soft tissues, spleen, adrenals, vertebral column, transverse colon, and stomach.
- **Lymphatic spread:** Through lymphatics it spreads to peripancreatic, gastric, mesenteric, omental, and portahepatic lymph nodes.
- **Blood spread:** It may spread to the liver, lungs, adrenal, and bones.

Clinical Features

Carcinoma head of pancreas: Present with obstructive jaundice.

Pancreatic cancers usually remain silent until they infiltrate into adjacent structures.

- **Pain:** It is usually the first and the **outstanding symptom**.
- **Obstructive jaundice:** It is associated with **carcinoma of the head** of the pancreas.

Trousseau's syndrome/migrating thrombophlebitis: Spontaneously appearing and disappearing (migratory) thrombosis.

Associated malignancies are:

1. Most common is carcinoma of pancreas
2. Carcinoma of lung
3. GI tract malignancies
4. Prostatic cancer
5. Ovarian cancer
6. Lymphoma.

- **Advanced stage:** Symptoms include: weight loss, anorexia, and generalized malaise and weakness.
- **Carcinomas of the body and tail of the pancreas:** It remains silent in the initial period.

Carcinoma of pancreas: May be associated with palpable gallbladder.

- **Migratory thrombophlebitis (Trousseau sign):** It is characterized by **spontaneously appearing and disappearing (migratory) thrombosis**. It is found in about 10% of patients. It is attributable to the production of platelet-aggregating factors and procoagulants from the carcinoma or its necrotic products.

Trousseau's sign: Carpopedal spasm in hypocalcemia.

Troisier's sign: Palpable left supraclavicular lymph node (Virchow's node).

Laboratory Findings

Raised serum levels of many enzymes and antigens (e.g. **carcinoembryonic antigen** and **CA19-9 antigen**) are often found in pancreatic cancer. These markers are nonspecific and lack the sensitivity. However, they are useful in follow-up of a patient's response to treatment.

Imaging techniques: Many imaging techniques, such as endoscopic ultrasonography and computed tomography, are useful for the diagnosis, but are not useful as screening tests.

Prognosis: The course is brief and progressive.

Carcinoma of head of the pancreas: Preferred test is contrast-enhanced CT scan.

SPINK1 is only associated with hereditary pancreatitis but not in pancreatic cancer.

Ductal adenocarcinoma of pancreas: Most common in the head of the pancreas.

Pancreatic carcinoma:

1. CA19-9 is the gold standard tumor marker.
2. CT scan-best test.

DIABETES MELLITUS

Diabetes mellitus is a **group of metabolic disorders having features of hyperglycemia**.

The prevalence of diabetes is increasing sharply in the developing countries because of more sedentary lifestyles. India and China have the largest prevalence of diabetics.

Diagnosis (Table 20.2)

Normal fasting blood glucose level: 70–120 mg/dL.

TABLE 20.2: Levels of blood glucose in normal, prediabetes and diabetes

	Euglycemic	Prediabetes	Diabetes
Fasting glucose level	Less than 100 mg/dL	Greater than 100 mg/dL but less than 126 mg/dL	Greater than 126 mg/dL on more than one occasion
OGTT	Less than 140 mg/dL	Greater than 140 mg/dL but less than 200 mg/dL	Greater than 200 mg/dL

Normally, the **blood glucose levels** are maintained in a very narrow range of **70–120 mg/dL**. The various definitions according to the American Diabetes Association (ADD) are as follows:

1. **Euglycemic:** Individuals are considered to be euglycemic, when:
 - **Fasting glucose level is less than 100 mg/dL**, or
 - **Glucose level less than 140 mg/dL** following an OGTT.
2. **Impaired glucose tolerance (prediabetes):** It is defined as condition in which there is **impaired glucose tolerance**, but **elevated blood sugar does not reach the criterion accepted for an outright diagnosis of diabetes**. The criteria for diagnosis are:

Prediabetes: Impaired glucose tolerance, but elevated blood sugar does not reach the criterion accepted for diagnosis of diabetes.

- A fasting plasma glucose between 100 and 125 mg/dL (“impaired fasting glucose”)
- 2-hour plasma glucose between 140 and 199 mg/dL following a 75-gm glucose OGTT, and/or
- A glycated (/glycosylated) hemoglobin (Hb_{A1C}) level between 5.7% and 6.4%.

Risks in prediabetes: (1) Progression to frank diabetes over time and (2) cardiovascular disease.

3. **Diabetes:** Any one of three criteria can be used for the diagnosis of diabetes:
 - A **fasting glucose level greater than 126 mg/dL**.
 - A random plasma glucose ≥ 200 mg/dL (in a patient with classic hyperglycemic signs).
 - 2-hour plasma glucose ≥ 200 mg/dL during an oral glucose tolerance test (OGTT) with a loading dose of 75 gm, and
 - A glycated hemoglobin (Hb_{A1C}) level $\geq 6.5\%$.

Classification

Q. Classify diabetes mellitus.

Diabetes is classified according to etiopathogenesis into different groups (Box 20.2) but **majority of cases fall into one of two broad classes namely: type 1 and type 2**.

TYPE 1 DIABETES

- Type 1 diabetes (T1D) Accounts for ~ 5–10% of all cases.
- **Age:** Most common in **childhood (younger than 20 years of age)**. Since, it can develop at any age, the term “juvenile diabetes” should be avoided.

BOX 20.2: Classification of diabetes mellitus

1. **Type 1 diabetes:** Immune-mediated or idiopathic
2. **Type 2 diabetes**
3. **Genetic defects of β -cell function:** Maturity-onset diabetes of the young (MODY), neonatal diabetes
4. **Genetic defects in insulin action**
5. **Exocrine pancreatic defects:** Chronic pancreatitis, hemochromatosis
6. **Endocrinopathies:** Acromegaly, Cushing syndrome, hyperthyroidism, pheochromocytoma
7. **Infections:** Cytomegalovirus, coxsackie B virus
8. **Drugs:** Glucocorticoids, thyroid hormone
9. **Genetic syndromes associated with diabetes:** Down syndrome, Klinefelter syndrome, Turner syndrome
10. **Gestational diabetes mellitus.**

Cause

- **Autoimmune disease** characterized by:
 - **Pancreatic β -cell destruction**
 - **Absolute deficiency of insulin.**
- **Idiopathic:** It is a rare form in which there is no evidence for autoimmunity.

Pathogenesis of Type 1 Diabetes Mellitus (Fig. 20.8)

Q. Write short note on pathogenesis of type 1 diabetes mellitus.

Involves interplay of both genetic susceptibility and environmental factors.

- **Genetic susceptibility:**
 - Incidence of type 1 diabetes is greater in twins of affected individuals than in the general population, and greater in monozygotic than in dizygotic twins.
 - **HLA genes:** About 95% of patients with type 1 diabetes have either human leukocyte antigen (HLA)-**DR3 or HLA-DR4, or both**, compared with the general population.

Type 1 diabetes: HLA-DR3 and HLA-DR4. Most important locus is the HLA gene cluster on chromosome 6p21.

- **Non-HLA genes:** It includes polymorphism in
 - ♦ Gene coding insulin.
 - ♦ *CTLA4* and *PTPN22* genes (involved in immune tolerance) are associated with excessive T-cell activation.
 - ♦ CD25.
- **Environmental factors:**
 - **Viral infections:** They may trigger islet cell destruction and associations have been found between

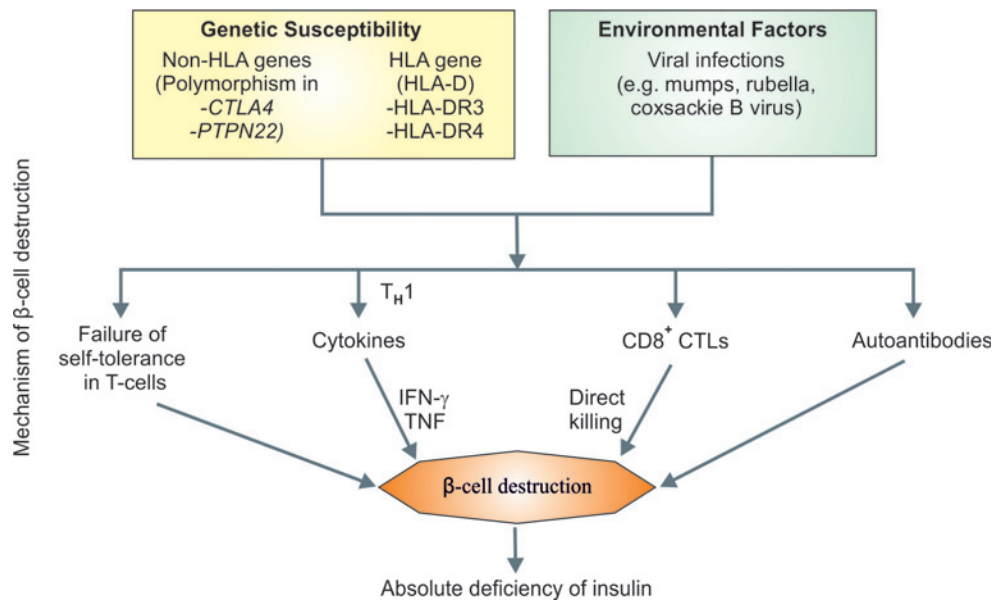


Fig. 20.8: Pathogenesis of type 1 diabetes mellitus

type 1 diabetes and infection with *mumps*, *rubella*, *coxsackie B*, or *cytomegalovirus*. Three different mechanisms explain the role of viruses in the induction of autoimmunity.

1. **Release of hidden or sequestered antigens:** Viral infections cause islet injury and inflammation, thereby release the sequestered β -cell antigens and activates auto-reactive T-cells.
2. **Molecular mimicry:** Viruses can produce proteins that mimic β -cell antigens. The immune response produced against the viral protein may cross-reacts with the self-tissue (β -cell antigens).
3. **Sharing of antigen epitopes:** First viral infections by a predisposing virus, during early in life might persist in the β -cells. Subsequent re-infection with a related virus known as precipitating virus, that shares antigenic epitopes may leads to an immune response against the β -cells.

Type 1 diabetes (T1D):

1. Autoimmune disease involving autoreactive T-cells and autoantibodies.
2. Destruction of β -cells \rightarrow absolute deficiency of insulin.

Mechanisms of β -Cell Destruction

The autoimmune damage starts many years before the disease becomes clinically evident. **Hyperglycemia and ketosis occur after more than 90% of the β -cells have been destroyed.** Various mechanisms include:

1. **Failure of self-tolerance in T-cells:** It is the **basic abnormality** in type 1 diabetes.
 - **Cause:** Failure of self-tolerance may be due to combination of:
 - Defective clonal deletion of self-reactive T-cells in the thymus.
 - Defects in the functions of regulatory T-cells.
 - Resistance of effector T-cells to suppression by regulatory cells.
2. **Through cytokines:** T_H1 cells may destroy β -cells by secreting cytokines, including IFN- γ and TNF.
3. **Directly by cytotoxic T-cells:** The $CD8^+$ CTLs may directly kill β -cells.
4. **Autoantibodies against β -cell antigens** (e.g. islet antigens β -cell enzymes): They are found in 70–80% of patients with type 1 diabetes, and in asymptomatic family members. It is not known whether autoantibodies cause injury or are result of islet injury.

Self-tolerance: Absence of immune response to an individual's own antigens. It is responsible for our ability to live in harmony with our cells and tissues:

Mechanism of β -Cell destruction:

1. Failure of self-tolerance
2. Through cytokines
3. Cytotoxic T-cells
4. Autoantibodies.

Type 1 DM: Autoantibodies include anti-insulin, anti-GAD, anti-ICA512.

TYPE 2 DIABETES

Type 2 diabetes (T2D): Multifactorial disease caused by insulin resistance and dysfunction of β -cells \rightarrow relative deficiency of insulin.

- Accounts for ~ **90–95%** of diabetic patients.

Q. Write short note on pathogenesis of type 2 diabetes mellitus.

- Majority** of patients are **overweight**.
- Though known as “adult-onset,” it is now found in children and adolescents.

Pathogenesis of Type 2 Diabetes Mellitus (Fig. 20.9)

Type 2 diabetes (T2D): Obesity has an important role in the development of insulin resistance.

Type 2 diabetes is a **multifactorial disease**.

- Environmental factors** play a role and includes:
 - Sedentary lifestyle.**
 - Dietary habits and associated obesity** particularly central or visceral obesity.
- Genetic factors:**
 - Type 2 diabetes has a **concordance rate of 35–60% in monozygotic twins compared with 17–30% in dizygotic twins.**

- Lifetime risk** for type 2 diabetes in an offspring is more than double if **both parents** are affected.
- Daibetogenic genes:** They have been found.
- There is **no evidence of an autoimmune basis**.

Metabolic Defects in Type 2 Diabetes

Type 2 diabetes: Insulin resistance develops before hyperglycemia and is usually associated with compensatory hyperfunction of β -cell and hyperinsulinemia.

Two important metabolic defects are:

Metabolic defects in type 2 diabetes:

- Insulin resistance
- β -cell dysfunction.

Insulin Resistance

Definition: Insulin resistance is the **decrease/failure of target (peripheral) tissues to insulin action**. Main factors in the development of insulin resistance is **obesity**.

- Obesity and insulin resistance:** Obesity is associated with type 2 diabetes and the **visceral obesity is found in more than 80% of patients**.
 - Amount of fat:** Insulin resistance is found even in simple obesity without hyperglycemia. The **risk for diabetes increases as the body mass index** (a measure of body fat content) **increases**.

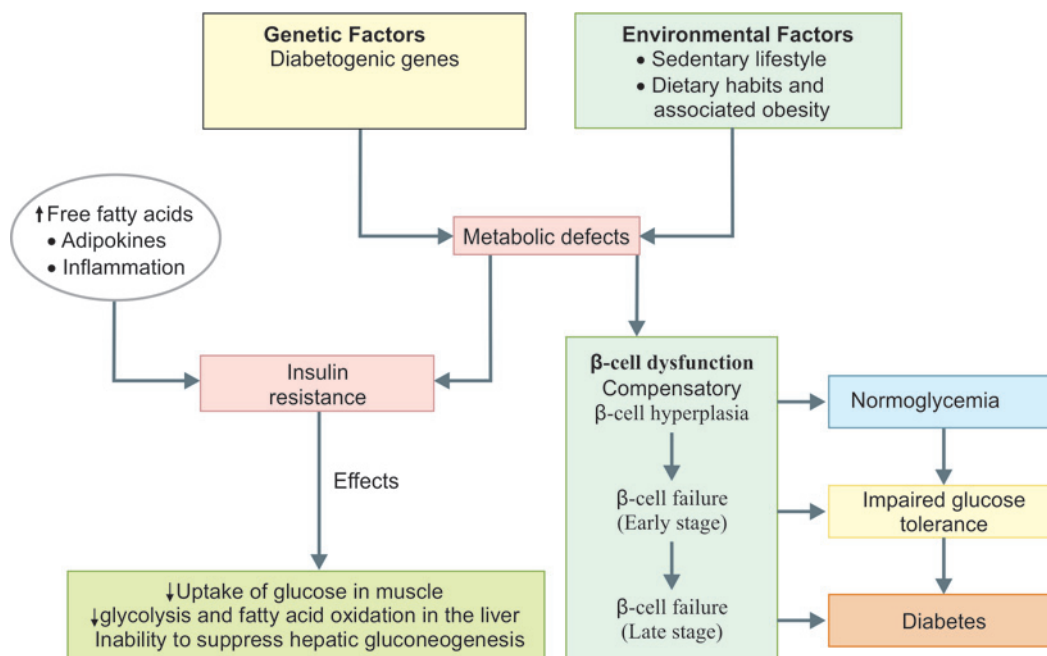


Fig. 20.9: Pathogenesis of type 2 diabetes. Insulin resistance associated with obesity is induced by free fatty acids, adipokines, and chronic inflammation in adipose tissue. Insulin resistance causes β -cells of pancreas to undergo compensatory hyperplasia and the resulting hypersecretion of insulin maintains normoglycemia. However, at some point, β -cell compensation is followed by β -cell failure, and diabetes develops

- **Distribution of body fat:** It has an effect on insulin sensitivity. **Central obesity** (abdominal fat) is **more associated with insulin resistance than are peripheral** (gluteal/subcutaneous) fat depots.

Insulin resistance: Failure of target tissues to respond normally to insulin.

Central obesity is associated with type 2 diabetes.

- **Causes of insulin resistance in obesity:** It is induced by (i) nonesterified free fatty acids, (ii) adipokines, (iii) chronic inflammation in adipose tissue, and (iv) activation of PPAR γ .
- **Excess of free fatty acids (FFAs):** There is an **inverse correlation between fasting plasma FFAs and insulin sensitivity**.
 - ◆ Obese individuals have excess circulating FFAs \rightarrow gets deposited as triglycerides in muscle and liver tissues \rightarrow results in markedly increased level of intracellular triglycerides.
 - ◆ Central adipose tissue is more “lipolytic” than peripheral sites, and central obesity is associated with insulin resistance.
 - ◆ Excess intracellular FFAs \rightarrow increases the fatty acid oxidation pathways \rightarrow leads to accumulation of cytoplasmic toxic intermediates such as diacylglycerol (DAG) and ceramide \rightarrow reduce the insulin signaling, thereby increases gluconeogenesis (insulin normally inhibits hepatic gluconeogenesis).
- **Adipokines:** Adipose tissue acts as a functional endocrine organ. It **secretes variety of proteins** into the systemic circulation, which are **termed adipokines** (or adipose cytokines). Adipokines can be divided into:
 - ◆ Prohyperglycemic adipokines, for example resistin, retinol binding protein 4 (RBP4).
 - ◆ Antihyperglycemic adipokines: Leptin and adiponectin improve insulin sensitivity. In obesity, adiponectin levels are reduced, which contributes to insulin resistance.
- **Inflammation:** Proinflammatory cytokines are secreted as a response to excess FFAs and glucose. They cause both insulin resistance and β -cell dysfunction. Excess FFAs within macrophages and β -cells activate the inflammasome (a multiprotein cytoplasmic complex) and secrete cytokine interleukin IL-1 β . IL-1 β secretes additional proinflammatory cytokines from macrophages, islet cells, and other cells and release into the circulation. IL-1 and other cytokines act on the major sites of insulin action and produce insulin resistance. Thus, excess FFAs can directly disturb insulin signaling within peripheral

tissues and also indirectly through the secretion of proinflammatory cytokines.

Monogenic diabetes: Mutation of PPARG.

Type 2 Diabetes (T2D): Obesity causes insulin resistance by:

1. Increasing FFAs
2. Reducing antihyperglycemic adipokines
3. Secreting proinflammatory cytokines which increase cell stress.

β -Cell Dysfunction

In type 2 diabetes, β -cell dysfunction manifests as **inadequate insulin secretion by the pancreatic β -cells (relative insulin deficiency)** in association with insulin resistance and hyperglycemia. β -cell dysfunction is multifactorial in origin.

• Obesity and β -cell dysfunction:

- **Compensatory β -cell hyperplasia:** Pancreatic β -cells **initially respond** to long-term demands of peripheral insulin resistance **by undergoing compensatory hyperplasia leading to increased insulin secretion (hypersecretion)**. Thus, the insulin secretion is initially higher for each level of glucose than in controls. This hyperinsulinemic state can compensate for peripheral resistance and maintain normal blood glucose for years.
- **β -cell failure (early stage):** However, at some point, β -cells exhaust their capacity to adapt. β -cell compensation **cannot maintain normal blood sugar level**. This stage, the patient develops impaired glucose tolerance.
- **β -cell failure (late stage):** The early stage of β -cell failure is followed by decreased insulin secretion, hyperglycemia and frank diabetes develops.
- **Molecular mechanisms of β -cell dysfunction:**
 - **Excess FFAs and decreased insulin signaling (lipotoxicity):** It may be responsible for both insulin resistance and β -cell failure.
 - **Chronic hyperglycemia (“glucotoxicity”).**
 - An abnormal “incretin effect,” with reduced secretion of hormones that promote insulin release (e.g. GIP and GLP-1).
 - **Direct toxicity by amyloid deposits:** In long-standing type 2 diabetes, amyloid deposits in islets is seen in more than 90% of cases. The islet **amyloid protein may be directly cytotoxic to islets** causing β -cell dysfunction.

β -cell dysfunction in DM: Initially compensatory β -cell hyperplasia followed by β -cell failure.

PATHOGENESIS OF THE COMPLICATIONS OF DIABETES

Q. Write short note on pathogenesis of complications in diabetes mellitus.

Pathogenesis is multifactorial and includes:

- Hyperglycemia (glucotoxicity) is the main mediator
- Insulin resistance (described already)
- Obesity (described already).

Hyperglycemia

Glycosylated hemoglobins (Hb_{A1C}): Marker of glycemic control.

- **Control of blood sugar level** (glycemic control) can **reduce the long-term complications** of diabetes.
- **Glycemic control is assessed** by estimation of **glycosylated hemoglobins (Hb_{A1C})**. Hb_{A1C} is formed by addition of glucose to hemoglobin in red cells. Hb_{A1C} should be maintained below 7% in diabetic patients and its **measurement is helpful in knowing the glycemic control over the lifespan of a red cell** (120 days).

Organ Damage by Hyperglycemia

- The chronic hyperglycemia and the metabolic disorders cause secondary **damage in multiple organ systems**.

- Common organs damaged are **kidneys** (end-stage renal disease), **eyes** (adult-onset blindness), **nerves**, and **blood vessels** (gangrene of lower extremity).

Diabetes mellitus: Good glycemic control prevents complications.

Effects Hyperglycemia

Harmful effects of persistent hyperglycemia on peripheral tissues can be brought out by **three distinct metabolic pathways**.

Formation of Advanced Glycation End Products (AGEs) (Fig. 20.10)

In diabetics, glucose binds to proteins nonenzymatically and is termed **nonenzymatic glycosylation**.

- **Initial products:** The initial products of nonenzymatic glycosylation are chemically known as Schiff bases **are labile and can dissociate rapidly**.

AGEs formation is markedly increased in the presence of hyperglycemia.

- **Later products:** The initial labile products form → **stable advanced glycation end products (AGEs)**. **The rate of AGE formation is markedly increased in the presence of hyperglycemia.**

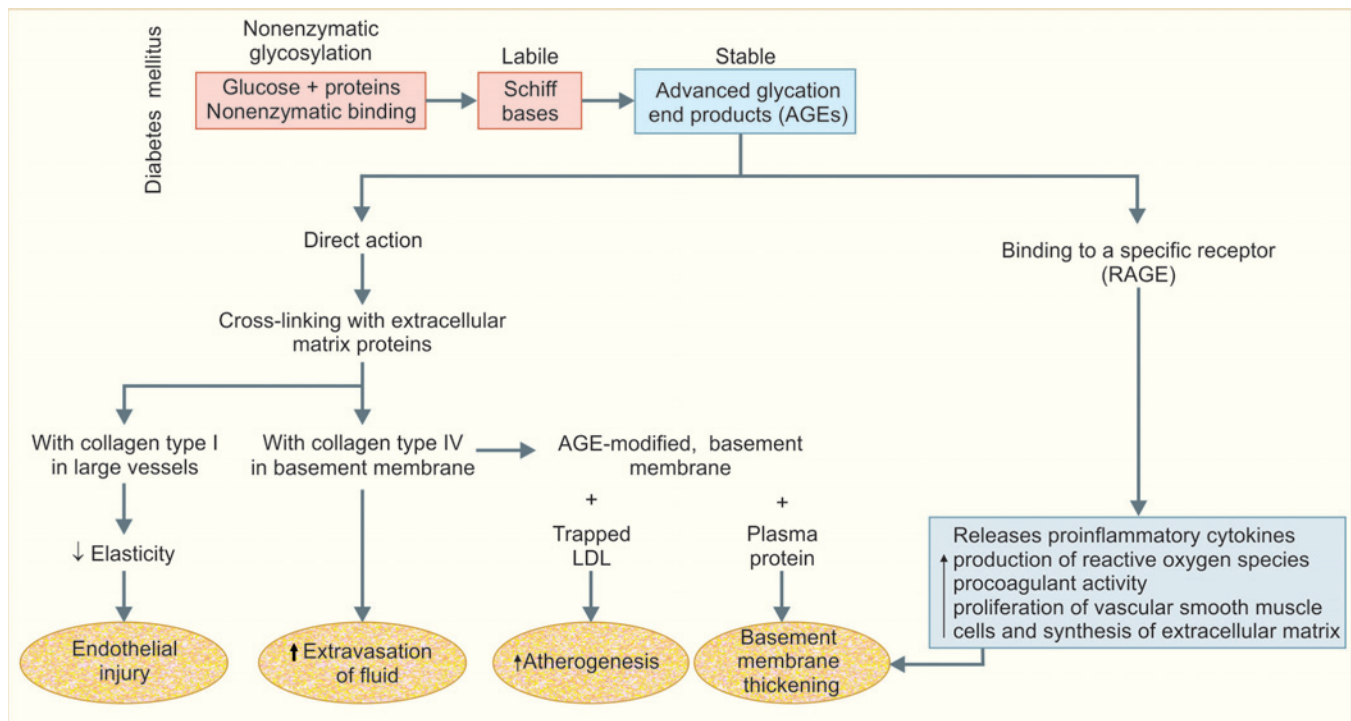


Fig. 20.10: Pathogenetic mechanism of injury advanced glycation end products (AGEs) in diabetes mellitus

Abbreviation: RAGE, Receptor for; AGE, LDL, Low-density lipoproteins

- **Effects of AGEs:** It may be brought out by binding of AGEs to its specific receptor (RAGE-receptor for AGE) or by its direct action.
 - **Through RAGE:** AGEs binds to RAGE on inflammatory cells (macrophages and T-cells), endothelial cells, and vascular smooth muscle cells. In the vascular compartment, AGE-RAGE has following actions:
 - ◆ Releases proinflammatory cytokines and **growth factors** from intimal macrophages.
 - ◆ Production of reactive oxygen species in endothelial cells.
 - ◆ **Increases procoagulant activity** on endothelial cells and macrophages.
 - ◆ **Increases proliferation of vascular smooth muscle cells** and synthesis of extracellular matrix.
 - **Direct effect of AGE:** AGEs can **directly cross-link extracellular matrix proteins** and its consequences are:
 - ◆ Resist proteolytic digestion → results in decreased removal and increased deposition of protein → **trapping of proteins in the glycosylated collagen of the basement membrane** → **basement membrane thickening**.
 - ◆ **Trapping of LDL in the intima** → **accelerating atherogenesis**.
- **Profibrogenic factors** (e.g. TGF- β) → results in **increased production of extracellular matrix and basement membrane material**.
- Plasminogen activator inhibitor (PAI-1) → **reduces fibrinolysis** and **favors vascular occlusion** (by forming atherosclerotic plaques or thrombus).

DM-effects of hyperglycemia:

1. Formation of AGEs
2. Activation of PKC
3. Disturbances in polyol pathways.

DM-Consequences of PKC activation:

- Neovascularization by VEGF
- Basement membrane thickening by TGF- β
- Reduced fibrinolysis and vascular occlusion by PAI-1.

Disturbances in Polyol Pathways

DM-Consequence of disturbance in polyol pathway: Increases susceptibility of cells to oxidative stress.

- **Some tissues** (e.g. nerves, lenses, kidneys, blood vessels) **do not require insulin for glucose transport**.
- **Persistent hyperglycemia increases the intracellular glucose in these tissues** → **excess intracellular glucose is metabolized by the enzyme aldose reductase** → **to sorbitol (polyol)** → to fructose.
- This **reaction uses NADPH** (the reduced form of nicotinamide dinucleotide phosphate) as a cofactor.
- NADPH is also necessary for the regeneration of reduced glutathione (GSH). GSH protects against injury by free radicals.
- **Reduced NADPH** → decrease in GSH → increases cells **susceptibility to oxidative stress**.

Persistent hyperglycemia causes diabetic neuropathy (*glucose neurotoxicity*).

TABLE 20.3: Effects of cross-linking of AGEs in the vasculature

Cross-linking with	Effects
• Collagen type I in large vessels	Decreases elasticity and predispose them to tear and endothelial injury
• Collagen type IV in basement membrane	Decreases endothelial cell adhesion and increases extravasation of fluid

DM-effects of AGEs on vessel:

1. Through RAGE
 - Endothelial injury
 - Increased ECM
2. Direct effect
 - Increased atherogenesis
 - Basement membrane thickening.

Activation of Protein Kinase C (PKC)

In patients with hyperglycemia, **intracellular hyperglycemia stimulates synthesis of diacylglycerol (DAG) from glycolytic intermediates**. DAG activates intracellular protein kinase C.

Consequences of PKC activation: It produces:

- **Proangiogenic molecules** such as **vascular endothelial growth factor (VEGF)** → causes **neovascularization** characteristic of **diabetic retinopathy**.

MORPHOLOGY

Pancreas in DM: Lesions are not diagnostic.

- Reduce number and size of islets
- Insulinitis
- Amyloid deposition in islets.

Pancreas

Lesions of pancreas are **not diagnostic** and are more common with type 1 than with type 2 diabetes. The morphological changes include:

- **Reduced number and size of islets:** It is seen in type 1 diabetes which is mild in type 2 diabetes.
- **Infiltration of islets (insulinitis):** Mainly by T lymphocytes predominantly in type 1 diabetes.

- **Amyloid deposition within islets:** It is observed in and around capillaries and between cells in type 2 diabetes. In advanced stages, the islets may be virtually obliterated and may show fibrosis.
- **Increase in the number and size of islets:** It may be seen in nondiabetic newborns of diabetic mothers as a hyperplastic in response to the maternal hyperglycemia.

Blood Vessels

- **Hyaline arteriosclerosis:** It can be found in hypertension, elderly nondiabetics without hypertension and more severe degree in diabetics also.
- In diabetics, it is **related to the duration of diabetes and the level of blood pressure.**
- It is characterized by **amorphous, hyaline thickening of the wall of the arterioles**, which may narrow the lumen of the vessel (refer Fig. 14.8A).
- **Diabetic microangiopathy:** It is characterized by **diffuse thickening of basement membranes**, which is **mostly seen in the capillaries of the skin, skeletal muscle, retina, renal glomeruli, and renal medulla.**
- Though capillaries show basement membrane thickening, **they are leakier to plasma proteins than normal.**
- The microangiopathy results in diabetic nephropathy, retinopathy, and some forms of neuropathy.

Hyaline arteriosclerosis:

- Diabetes mellitus
- Hypertension
- Elderly nondiabetic without hypertension.

Diabetic microangiopathy: Diffuse thickening of capillaries mostly seen in skin, skeletal muscles, retina and kidney.

Renal changes in diabetes mellitus are discussed in page no. 614-615.

Clinical Features of Diabetes (Fig. 20.11)

Clinical features of diabetes: Classical triad—

1. Polydipsia
2. Polyphagia
3. Polyuria.

Type 1 Diabetes Mellitus

- **Age:** Type 1 diabetes can occur at any age.
- **Classical classic triad of diabetes:** It consists of **polyuria, polydipsia, polyphagia**, and in severe cases **ketoacidosis**, are due to metabolic derangements.
- **Insulin requirement:** In the **initial 1 or 2 years**, the exogenous insulin required may be **minimal** because of endogenous insulin secretion → **later**, its requirement suddenly **increases**.

Consequences of Insulin Deficiency

Insulin is an anabolic hormone and its deficiency → results in a catabolic state, which affects glucose metabolism, fat and protein metabolism.

- **Carbohydrate metabolism:**
 - **Diminished transport of glucose into muscle cells and adipose tissue.**
 - **Reduction of stored glycogen in liver and muscle due to glycogenolysis** → this further aggravates the **hyperglycemia.**
 - When **hyperglycemia exceeds the renal threshold level** → develops **glycosuria** → leads to osmotic diuresis → **increased quantity of urine known as polyuria**, with **loss of water and electrolytes.**
 - Water loss through urine + hyperosmolarity (due to hyperglycemia) → causes **depletion of intracellular water** → **stimulates** the osmoreceptors of the **thirst centers** of the brain → results in increased thirst known as **polydipsia.**
- **Protein and fat metabolism:**
 - Insulin deficiency → causes **catabolism of proteins and fats** → produces a **negative energy balance** → leads to **increased appetite (polyphagia).**
 - In spite of increased appetite, catabolic effects insulin results in **paradoxical loss of weight and muscle weakness.**
- **Diabetic ketoacidosis:**
 - Serious complication of diabetes.
 - More **common and marked in type 1 diabetes**, but may also occur in type 2 diabetes.
 - **Mechanism:**
 - ♦ **Diuresis and dehydration:** Marked insulin deficiency + associated **epinephrine release** → stimulates the **secretion of glucagon** → leads to **decreased peripheral utilization of glucose + increased gluconeogenesis** → causes **severe hyperglycemia** (the blood glucose levels of 500–700 mg/dL) → **osmotic diuresis and dehydration** (characteristic features of ketoacidosis).

Diabetic ketoacidosis: Complication of type 1 diabetes mellitus.

- ♦ **Activation of the ketogenic machinery:**
 - ◊ Insulin deficiency → **stimulates lipoprotein lipase** → which **breaks down fat stores** → **increases the free fatty acids levels.**
 - ◊ **Free fatty acids** are esterified to fatty acyl coenzyme A in the liver.
 - ◊ In the mitochondria of liver cells, fatty acyl coenzyme A molecules are oxidized to **ketone bodies** (acetoacetic acid and β -hydroxybutyric acid).

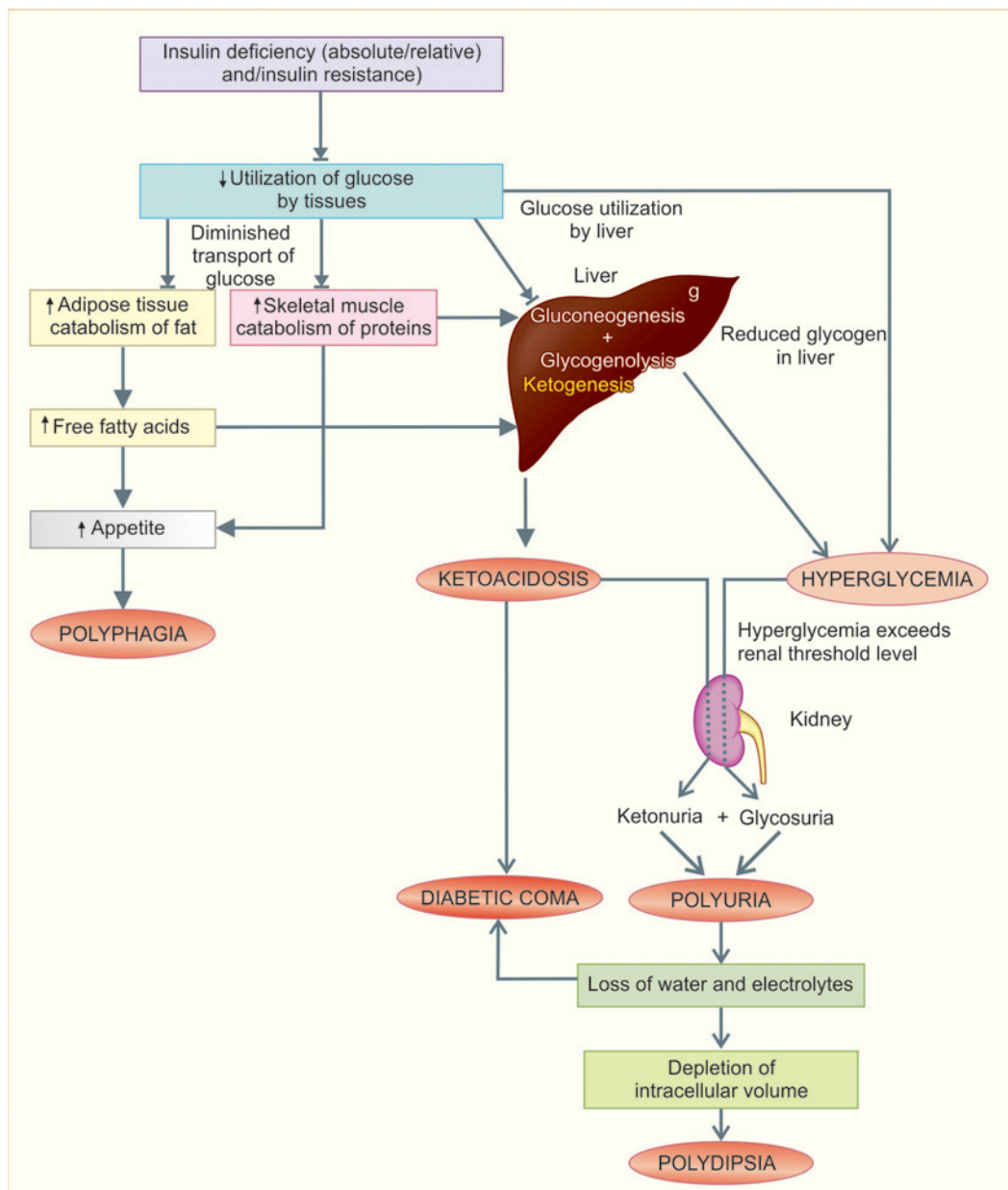


Fig. 20.11: Sequence of metabolic derangements underlying the symptoms and signs of uncontrolled hyperglycemia in diabetes mellitus

- ◊ When the **rate of production of ketone bodies exceeds the rate of utilization** by peripheral tissues, it results in **ketonemia** and **ketonuria**.
- ◊ If the excretion of ketone bodies in the urinary is compromised by dehydration, it results in **systemic metabolic ketoacidosis**.

Type 2 Diabetes Mellitus

- **Age:** Usually occurs in older **above the age of 40 years** and frequently in **obese individuals**. Due to increase in obesity and sedentary lifestyle, it is now detected also in children and adolescents.
- **Presentation:** **Polyuria, polydipsia, unexplained weakness or weight loss. Ketoacidosis is infrequent** and presentation is usually mild.
- In **asymptomatic individuals**, the **diagnosis is made after routine blood or urine testing**.

Differences between type 1 and type 2 diabetes mellitus are listed in Table 20.4.

TABLE 20.4: Differences between type 1 and type 2 diabetes mellitus**Q. List the differences between type 1 and type 2 diabetes mellitus.**

	Type 1 diabetes mellitus	Type 2 diabetes mellitus
Clinical		
Age of onset	Childhood and adolescence	Adult, also seen in childhood and adolescence
Weight	Normal or present with weight loss	Majority are obese
Insulin levels	Progressive decrease	Increased (early); normal or moderate decrease (late)
Circulating islet autoantibodies	Detected (anti-insulin, anti-GAD, anti-ICA512)	Not found
Diabetic ketoacidosis	Develops in absence of insulin therapy	Nonketotic hyperosmolar coma
Genetics		
Human leukocyte antigen (HLA) association	Major linkage to MHC (major histocompatibility complex) class I and II genes HLA-DR3 and/-HLA-DR4	No HLA association
Non-HLA genes	Polymorphisms in <i>CTLA4</i> and <i>PTPN22</i>	Diabetogenic and obesity-related genes
Etiopathogenesis		
Mode of development	Autoimmune	Multifactorial
Mechanism	Breakdown in self-tolerance to islet auto-antigens	Insulin resistance in peripheral tissues, β -cell dysfunction
Morphology		
Insulinitis (inflammation of islets)	Inflammatory infiltrate of T-cells and macrophages in islets	No insulinitis; amyloid deposition in islets
β -cell depletion	Moderate to severe	Mild

Complications (Fig. 20.12)

Q. Write short note on complications of type 1 diabetes mellitus.

- Complications are similar in both type 1 and type 2 diabetes.
- **Long-term complications of diabetes** usually develop about 15–20 years after the onset of hyperglycemia. They are responsible for the majority of the morbidity and mortality.

Complications of diabetes: Mainly affects blood vessels, kidney, eyes and nerves.

Most abundant glycoprotein present in basement membrane is: Laminin.

Diabetic Macrovascular Disease

The lesions of large- and medium-sized muscular arteries are the **most common causes of mortality** in long-standing diabetes. These include:

- **Atherosclerosis:** Diabetes is one of the major modifiable risk factor for atherosclerosis and other cardiovascular morbidities. The **atherosclerosis is more severe and occurs at earlier age**. Diabetics have **increased levels of plasminogen activator inhibitor (PAI-1)**, which **inhibits fibrinolysis and favors development of**

atherosclerotic plaques. Renal arteries also develop severe atherosclerosis.

- **Myocardial infarction:** It is due to atherosclerosis of the coronary arteries and is the **most common cause of death in diabetics**. Diabetics have greater risk of coronary artery disease and cardiovascular complications than nondiabetics. Risk for cardiovascular disease is more even in prediabetics.
- **Gangrene of the lower extremities:** It results from advanced vascular disease and is more common in diabetics.
- **Renal vascular insufficiency.**
- **Cerebrovascular accidents (stroke).**

Microvascular Disease (Microangiopathy)

It involves small vessels and is characterized by capillary dysfunction in target organs and is mainly observed in kidneys (nephropathy), retina (diabetic retinopathy) and peripheral nerves (neuropathy).

- **Diabetic nephropathy** (refer page 613-615): About **30–40% of all diabetics** develop nephropathy and is **leading cause of end-stage renal disease**. The different stages in diabetic nephropathy are:
 - **Microalbuminuria:** It is the **earliest manifestation** of diabetic nephropathy in which the urine has low

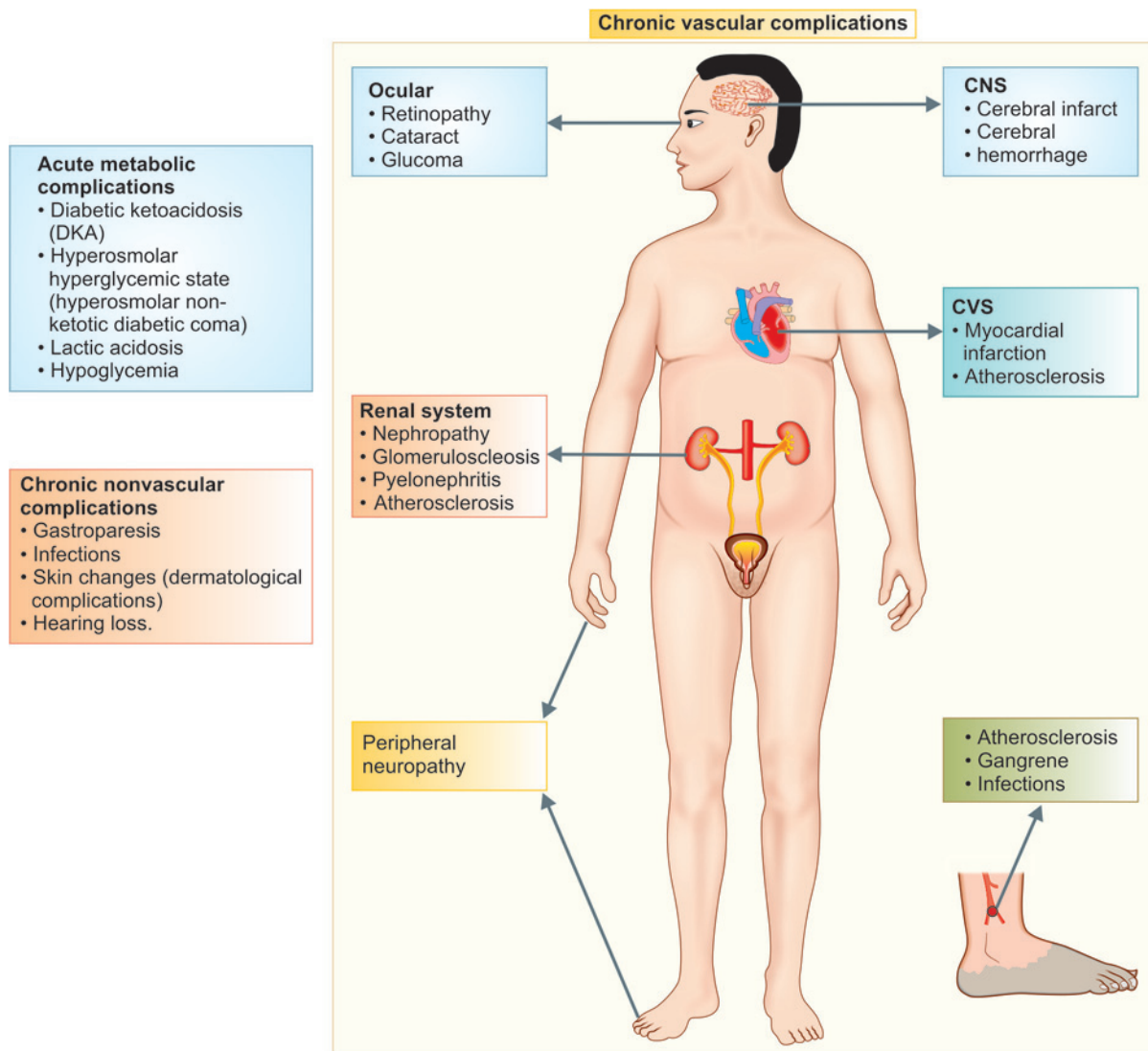


Fig. 20.12: Complications of diabetes

Abbreviation: CNS, central nervous system; CVS, cardiovascular system

amounts of albumin (>30 mg/day, but <300 mg/day). Microalbuminuria is also a marker for increased cardiovascular morbidity and mortality in either type 1 or type 2 diabetics.

- **Nephropathy with macroalbuminuria:** Without specific interventions, diabetics will develop **overt nephropathy with macroalbuminuria** (>300 mg of urinary albumin per day), usually associated with hypertension.
- **End-stage renal disease:** The overt nephropathy may progress to end-stage renal disease.
- **Diabetic retinopathy:** It develops in ~60-80% of diabetics, about 15-20 years after diagnosis. The basic

lesion of retinopathy is **neovascularization caused by hypoxia-induced overexpression of VEGF in the retina**. Diabetics have **increased risk for glaucoma and cataract formation**.

- **Diabetic neuropathy:** It may involve the **central and peripheral nervous systems**.
 - **Distal symmetric polyneuropathy:** It affects **both motor and sensory function** of the lower extremities later, it may involve the upper extremities leading to “glove and stocking” pattern of polyneuropathy.
 - **Autonomic neuropathy:** Produces **disturbances in bowel and bladder function** and sometimes sexual impotence.

- **Diabetic mononeuropathy:** It may manifest as sudden footdrop, wristdrop, or isolated cranial nerve palsies.

Microalbuminuria:

- Urine has low amounts of albumin (>30 mg/day, but <300 mg/day)
- Increased cardiovascular morbidity and mortality.

Diabetic Dyslipidemia

It is a condition in which there are **increased blood levels of triglycerides and LDL and decreased levels of the high-density lipoprotein** (protective). This develops due to insulin resistance, which causes increased production

of atherogenic lipoproteins by liver and decreased uptake of circulating lipids in peripheral tissues.

Increased Susceptibility to Infections

DM: Increased susceptibility to infections due to defective neutrophil functions.

Infections of the skin, tuberculosis, pneumonia, and pyelonephritis are common in diabetes. The increased susceptibility may be due to **defective neutrophil functions** (chemotaxis, adherence to the endothelium, phagocytosis, and microbicidal activity), and **impaired cytokine production by macrophages**.

Hyaline arteriosclerosis is discussed in pages 384-385.

Kidney and Urinary Tract Disorders

INTRODUCTION

Unit of kidney: The **nephron** (Fig. 21.1) is the **structural unit** of the kidney and consists of glomerulus and its tubule, and common collecting system.

Glomerulus (Figs 21.2 to 21.4)

- It is the **basic filtering unit** of the kidney that consists of a tuft of **anastomosing network of capillaries** formed by branching of the afferent arteriole.
- Capillaries are lined by **fenestrated (window-like openings) endothelium** lying on a **glomerular basement membrane (GBM)**. The outer portions of the fenestrated endothelial cell are in contact with the inner surface of

the GBM, whereas the central part is in contact with the mesangial cell and mesangial matrix.

- The entire **outer surface of GBM** is covered by **visceral epithelial cell** (podocyte) foot processes. The visceral epithelial cells line the glomerular side of Bowman's space, whereas the **parietal epithelial cells** line **Bowman's capsule** on the opposite side.
- **Mesangium:** It consists of **mesangial cells** and basement membrane-like **mesangial matrix** and supports the entire glomerular tuft.

Diseases of the kidney can be divided into four major groups depending on the basic morphologic components affected namely: glomerular diseases, tubules diseases, interstitial diseases, and diseases of blood vessels.

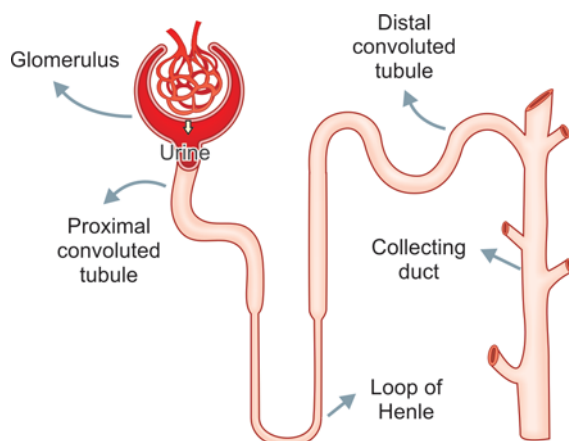


Fig. 21.1: Different components of nephron

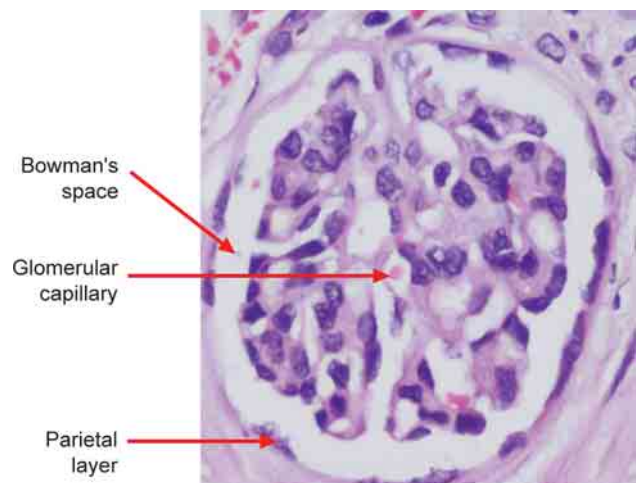


Fig. 21.2: Photomicrograph of light microscopic appearance of glomerulus

Clinical Manifestations of Renal Diseases (Table 21.1)

TABLE 21.1: Clinical manifestation of renal diseases

Disease	Manifestations
Glomerular syndromes	
Nephritic syndrome (glomerular disease)	Acute onset of either grossly visible hematuria (red blood cells in urine) or microscopic hematuria with dysmorphic red cells and red cell casts on urinalysis, diminished GFR, mild to moderate proteinuria , and hypertension
Rapidly progressive glomerulonephritis	Nephritic syndrome with rapid decline in GFR (within hours to days)
Nephrotic syndrome (glomerular disease)	Heavy/massive proteinuria (more than 3.5 g/day), hypoalbuminemia, severe edema, hyperlipidemia, and lipiduria (lipid in the urine)
Chronic kidney disease (previously termed chronic renal failure)	Persistently diminished GFR less than 60 mL/minute/1.73 m ² for at least 3 months, from any cause, and/or persistent albuminuria. It is the end result of all chronic renal parenchymal diseases
Asymptomatic hematuria or proteinuria, or both	Usually due to mild glomerular abnormalities
Others	
Azotemia is biochemical manifestation of kidney injury or extrarenal causes Uremia = azotemia + clinical signs and symptoms + biochemical abnormalities	Raised blood urea nitrogen (BUN) or an elevated serum creatinine due to reduced glomerular filtration rate (GFR) Renal causes: Acute or chronic kidney injury Extrarenal causes: Prerenal azotemia (e.g. due to hypotension or excessive fluid loss from any cause) or postrenal azotemia (due to obstruction to urine flow distal to the kidney)
Acute kidney injury	Rapid decline in GFR (within hours to days), with deranged fluid and electrolyte balance, and retention of metabolic waste products normally excreted by the kidney (urea and creatinine), oliguria or anuria (reduced or no urine flow) in most severe cases. It may be due to glomerular, interstitial, vascular or acute tubular injury
End-stage renal disease (ESRD)	GFR is less than 5% of normal and is the terminal stage of uremia
Renal tubular defects due to diseases that either directly affect tubular structures or cause defects in specific tubular functions	Polyuria (excessive urine formation), nocturia, and electrolyte disorders
Urinary tract obstruction and renal tumors	Features depends on anatomic location and nature of the lesion
Urinary tract infection. It may affect kidney (pyelonephritis) or the bladder (cystitis)	Bacteriuria and pyuria (bacteria and leukocytes in the urine) Infection may be symptomatic or asymptomatic
Nephrolithiasis (renal stones)	Spasms of severe pain (renal colic) and hematuria
Uremia: Azotemia + clinical signs and symptoms + biochemical abnormalities.	

GLOMERULAR DISEASES

Q. Classify glomerular diseases.

Glomerular diseases may be broadly divided into primary and secondary:

- **Primary glomerulonephritis or glomerulopathy** (those without inflammatory cells): Kidney is the only or predominant organ involved.
- **Secondary glomerular diseases:** In these disorders, glomeruli are involved secondary to a systemic disease. Most common forms of glomerulonephritis are listed in Box 21.1.

Clinical Manifestations

Clinical manifestations are grouped into the **five major glomerular syndromes** (Table 21.1).

Histological Alterations

Glomerulonephritis/glomerulopathies may show one or more of **three basic histological changes** namely: (1) increased cellularity, (2) basement membrane thickening, and (3) hyalinization or sclerosis.

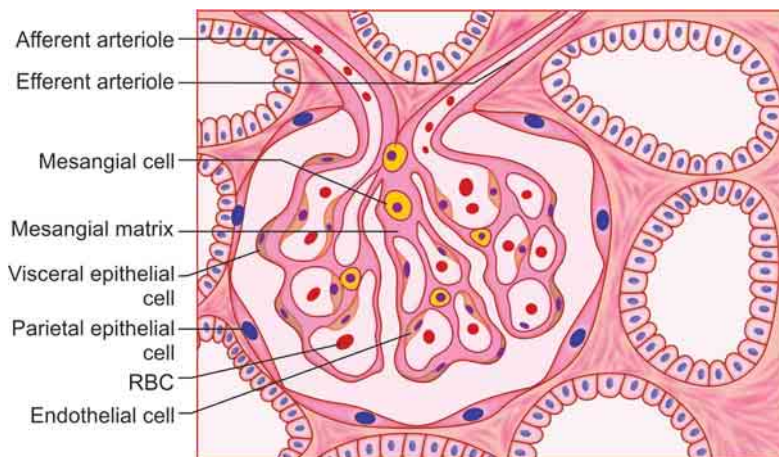


Fig. 21.3: Light microscopic appearance of glomerulus (diagrammatic)

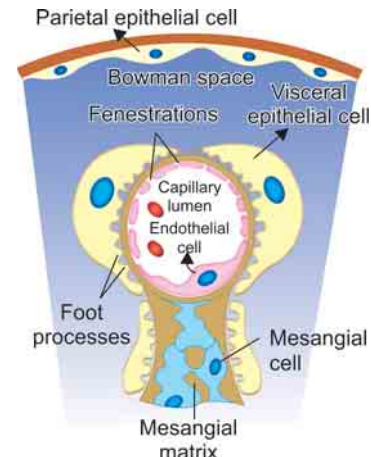


Fig. 21.4: Diagrammatic representation of electron microscopic view of normal renal glomerulus. The relationship of the different cell types to the glomerular basement membrane and mesangial matrix is illustrated using a single glomerular loop

BOX 21.1: Common glomerular diseases

Primary glomerulonephritis/glomerulopathies

Acute proliferative glomerulonephritis: Post-infectious, others
 Rapidly progressive (crescentic) glomerulonephritis (RPGN)
 Minimal-change disease (MCD)
 Membranous glomerulonephritis (MGN)
 Membranoproliferative glomerulonephritis (MPGN)
 Dense deposit disease
 Focal segmental glomerulosclerosis (FSGS)
 IgA nephropathy
 Chronic glomerulonephritis

Systemic diseases with glomerular involvement

Systemic immunological diseases: For example, Systemic lupus erythematosus
 Metabolic diseases: For example, Diabetes mellitus
 Vasculitis: Microscopic polyarteritis/polyangiitis, Wegener granulomatosis, Henoch-Schönlein purpura
 Amyloidosis
 Goodpasture syndrome
 Bacterial endocarditis

Hereditary disorders

Alport syndrome
 Thin basement membrane disease
 Fabry disease

- **Cellular proliferation:** It may involve any of the cells of glomeruli (mesangial or endothelial or epithelial cells).
- **Leukocytic infiltration:** These include neutrophils, monocytes, and lymphocytes.
- **Formation of crescents:** These are formed due to **proliferation of parietal epithelial cells and infiltration by leukocytes** (refer Fig. 21.10). This develops as a **response to fibrin that leaks into the urinary space through ruptured basement membranes**.

Crescentic: Extensive proliferation of parietal epithelial cells and leukocyte infiltration.

Basement Membrane Thickening

It may be due to:

- **Deposition of amorphous electron-dense** (immune complexes) **material:** The deposits may be on the **endothelial or epithelial side GBM or within the GBM itself**.
- **Thickening of the basement membrane:** For example, in **diabetic glomerulosclerosis**.

Hyalinosis and Sclerosis

- **Hyalinosis:** It is the **accumulation of homogeneous and eosinophilic material in the glomeruli** and consists of **plasma proteins** that have leaked from the circulation into glomerular structures. It is usually due to endothelial or capillary wall injury.
- **Sclerosis:** It is characterized by **accumulations of extra-cellular collagenous matrix**, either in the **mesangium** (e.g. diabetic glomerulosclerosis) or in the **capillary loops, or both**.

Increased Cellularity (Hypercellularity)

It is characterized by an **increase in the number of cells in the glomerular tufts**. Such lesion is called **proliferative glomerulonephritis**. Hypercellularity may be due to:

BOX 21.2: Terms used in glomerulopathies/glomerulonephritis

Diffuse or generalized: A lesion affecting/involving all of the glomeruli in the kidney

Global: Involving the whole/entire individual glomeruli

Focal: A lesion involving only a some/proportion of the glomeruli in the kidney but leaving others unaffected

Segmental: Affecting a part/segment of each glomerulus

Capillary loop or mesangial: Affecting predominantly capillary or mesangial regions.

Hyalinosis:

- Accumulation of homogeneous and eosinophilic material in the glomeruli
- Consists of plasma proteins.

Sclerosis: Accumulations of extracellular collagenous matrix, either in the mesangium or capillary loops or both.

The cause in majority of the primary glomerulopathies is not known and they are classified by their microscopic features.

Nomenclature of glomerular injury is used to describe the distribution/pattern of injury in glomeruli (Box 21.2).

PATHOGENESIS OF GLOMERULAR INJURY (FIG. 21.5)

Immune mechanisms are responsible for **most of primary** glomerulopathy and **many of the secondary** glomerular disorders (Box 21.3).

BOX 21.3: Various immune mechanisms involved in glomerular injury

1. Antibody-mediated injury

In situ immune complex deposition

- Fixed intrinsic tissue antigens: For example, anti-GBM nephritis
- Planted antigens
 - Exogenous: For example, infectious agents, drugs
 - Endogenous: For example, DNA, nuclear proteins

Circulating immune complex deposition

- Endogenous antigens: For example, DNA, tumor antigens
- Exogenous antigens: For example, infectious products

2. Cell-mediated immune injury

3. Activation of alternative complement pathway

Abbreviation: GBM, glomerular basement membrane.

ANTIBODY-MEDIATED INJURY

Antibody-mediated glomerular injury: Important mechanism of glomerular damage.

Antibody-mediated glomerular injury: Most commonly caused by formation of immune (antigen-antibody) complexes (in situ or circulating).

Antibody-mediated glomerular injury can be produced by **two mechanisms**.

1. **In situ antibodies:** The antibodies may be against
 - **Fixed (intrinsic) glomerular antigens:** For example, antiglomerular basement membrane (anti-GBM) antibody-induced glomerulonephritis or
 - **Antigen** which are **planted** within the glomerulus.
2. **Circulating (antigen-antibody) complexes** deposition in the glomerulus.

In Situ Antibodies

- This type of injury is characterized by antibodies reacting directly with **intrinsic (fixed) tissue antigen, or antigens** which are “**planted**” in the glomerulus.

In Situ Antibodies against Fixed Antigen (Fig. 21.5)

- This is an **anti-GBM antibody-mediated glomerulonephritis**. In this type of injury, antibodies directly react with **intrinsic (fixed) tissue antigen** (that are normal components) of the GBM proper.
- Example in **humans** is **Goodpasture syndrome** and results from formation of **autoantibodies against fixed antigen present in the GBM**. This type of glomerulonephritis has an experimental counterpart known as Masugi or nephrotoxic nephritis.
- **Goodpasture syndrome:** It is characterized by a conformational changes in the α_3 chain of type IV collagen of GBM. This fixed altered GBM antigen (Goodpasture antigen) can incite autoimmunity and produce **anti-GBM antibodies against Goodpasture antigen**. These antibodies may sometimes cross-react with not only renal GBM but also with basement membrane of lung alveoli, results in simultaneous production of lung and kidney lesions and this is known as Goodpasture syndrome (refer pages 602-3).
- **Masugi or nephrotoxic nephritis:** It is an experimental model of nephritis produced in rats.

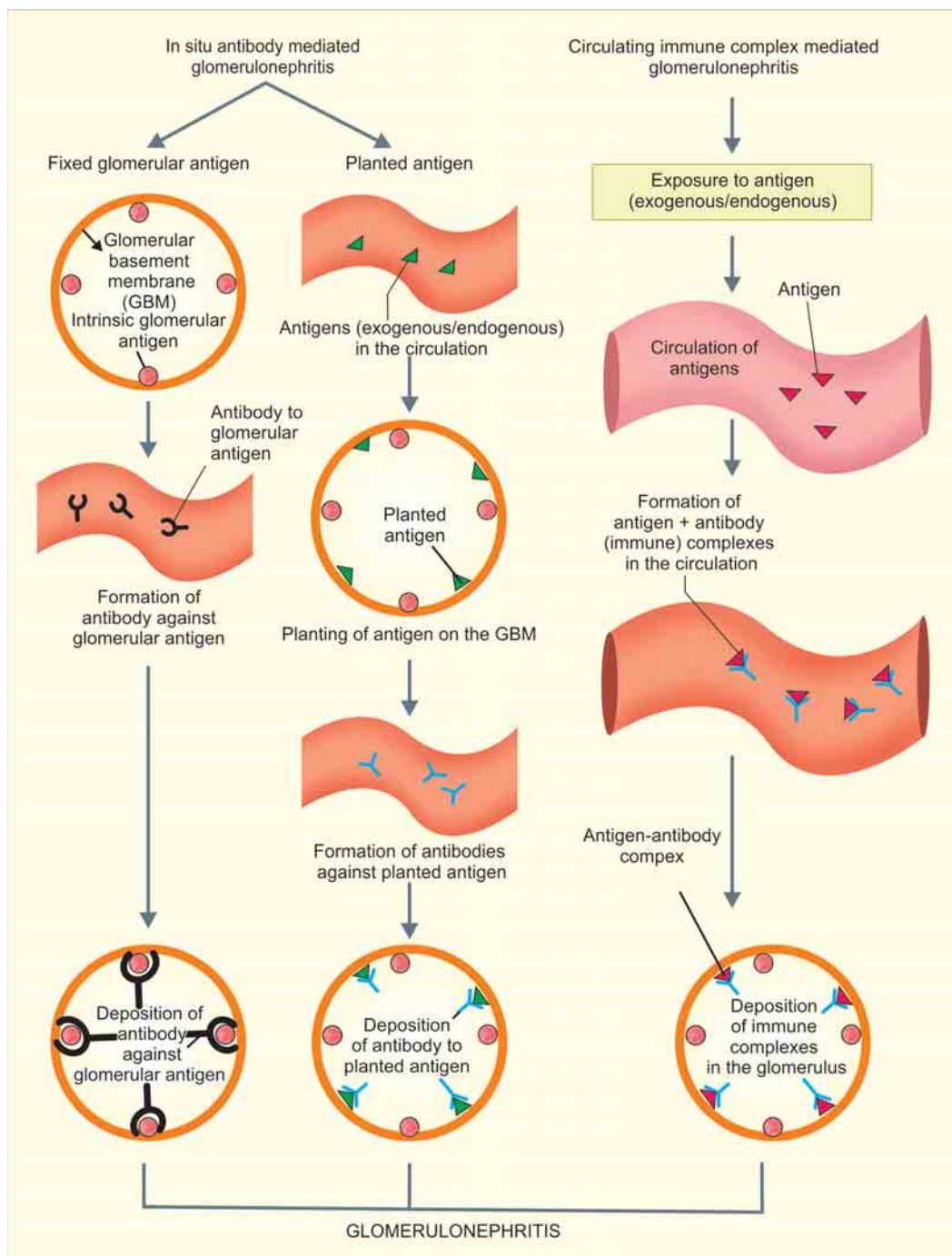


Fig. 21.5: Various immunological mechanisms of antibody mediated glomerulonephritis. Glomerulonephritis may develop to an antibody reaction against glomerular antigen (e.g. Goodpasture antigen) or to antibody reacting against foreign antigen that is planted in the basement membrane or circulating immune complexes that become deposited in the glomeruli

- First, rat kidney tissue is injected into the rabbits to produce anti-rat kidney antibodies (anti-GBM antibody).
- The anti-rat kidney antibodies from the rabbits injected into the rat.
- The injected anti-rat kidney antibodies bind along the entire length of the GBM and produces glomerular damage.
- Injected anti-rat kidney antibody (i.e. anti-GBM antibody) is rabbit immunoglobulin (Ig). In the rat,

this rabbit Ig is foreign protein and thus acts as an antigen eliciting anti-Ig antibody. The rat anti-Ig antibodies then react with the rabbit Ig. This further aggravates the glomerular damage.

- They produce **diffuse linear pattern of immunofluorescence**. This is in contrast to the granular lumpy pattern of immunofluorescent staining seen in circulating immune complexes (described below).

In Situ Antibodies against Planted Antigens

(Fig. 21.5)

- Some antigens may not be normally present in the glomerulus but are “planted” to the glomerulus.
- Such planted antigens may interact with various intrinsic components of the glomerulus.
- Planted antigens includes viral, bacterial, and parasitic products and drugs. Antibodies produced against these planted antigens can react in situ in the glomerulus.
- Immunofluorescence: They produce **granular fluorescence** under immunofluorescence microscopy, similar to the circulating immune complex nephritis.
- **Example** for this type of glomerulonephritis in humans is membranous nephropathy discussed in page 605.
- **Heymann nephritis**: It is experimental model for **in situ antibodies against planted antigens**.
 - It induced in the rat by immunizing it with an antigen contained within preparations of proximal tubular brush border. This antigen is called as **Heymann antigen** which is a **protein called megalin**.
 - The rat develops antibodies to this antigen, and antibody reacts with an antigen complex located on the basal surface of visceral epithelial cells. The rat develops a membranous nephropathy.
 - *Electron microscopy*: It shows numerous discrete electron-dense deposits along the **subepithelial aspect of the basement membrane**.

Circulating Immune Complex Glomerulonephritis

Immune complexes: Antigen may be exogenous or endogenous.

Most of glomerulonephritis are immune complex-mediated. This type of glomerulonephritis is caused due to **trapping of circulating antigen-antibody complexes (type III hypersensitivity reactions) within glomeruli**. The antibodies are not against any of glomerular constituents, and the immune complexes localize within the glomeruli.

Antigens: They may be of endogenous or exogenous origin.

- **Endogenous:** Autoimmune diseases (e.g. Systemic lupus erythematosus)

- **Exogenous:**

- **Microbial antigens:**

- ♦ Bacterial products (e.g. streptococci)
- ♦ Viral antigen (e.g. hepatitis B virus, hepatitis C virus antigens)

- **Spirochetal antigens** (of *Treponema pallidum*)

- **Parasitic:** *Plasmodium falciparum*

- **Tumor antigens**

- **Unknown antigen.**

Antibodies are produced against the antigens from **antigen-antibody complexes** in the **circulation** which gets **trapped in the glomeruli** and **produce injury**.

Electron Microscopy (Fig. 21.6)

EM: Immune complexes are electron dense.

It shows the immune complexes as electron-dense deposits (Fig. 21.6) that may be found in any of these sites:

- **Mesangium** (mesangial deposits).
- **Subendothelial deposits** between the endothelial cells and the GBM.
- **Subepithelial deposits** between the outer surface of the GBM and the podocytes.
- **Basement membrane.**
- **More than one site** mentioned above.

Immunofluorescence Microscopy

Immune complexes: Granular pattern of immunofluorescence.

The immune complexes appear as granular immunofluorescence deposits along the basement membrane, in the mesangium, or in both sites.

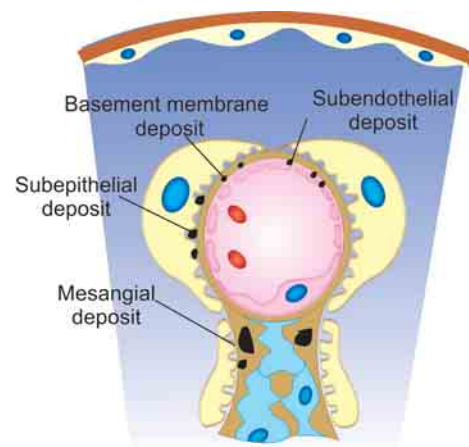


Fig. 21.6: Various locations of immune complex deposition in the glomerulus include: subepithelial, subendothelial, basement membrane and mesangial

Factors that Determine Localization

The localization of antigen, antibody, or immune complexes in the glomerulus mainly depend on: (1) the charge and (2) size of the molecules.

- **Molecular charge:**

GBM: Size and charge dependent filtration.

- **Highly cationic molecules** can cross the GBM and appear in the subepithelial location.
- **Highly anionic molecules** cannot cross the GBM and are trapped in subendothelial region.
- **Molecules of neutral charge** tend to accumulate in the mesangium.
- **Molecular size:** Large circulating complexes are removed from the circulation by the mononuclear phagocyte system and they do not enter the GBM in sufficient quantities. Thus, they **do not produce glomerulonephritis**. Immune complexes of medium size and with slight antigen excess are the most pathogenic.

Immune complexes: Activate complement → attracts leukocytes → damages tissues.

Cytotoxic antibodies directed against glomerular cell components may rarely cause glomerular injury.

Glomerular damage by activation of alternate pathway is discussed on page 611.

NEPHRITIC SYNDROME

Nephritic syndrome:

- Hematuria
- RBC casts/dysmorphic red cells in the urine
- Moderate proteinuria
- Oliguria
- Hypertension.

Definition: Nephritic syndrome is characterized by **hematuria** (gross or microscopic), dysmorphic red cells, **red cell casts** in the urine, **azotemia** and **oliguria** (due to decreased glomerular filtration rate), and **mild to moderate hypertension**. Proteinuria and edema are common, but they are not as severe as in the nephrotic syndrome.

One of the causes of nephritic syndrome is acute proliferative glomerulonephritis.

Acute Proliferative Glomerulonephritis

Proliferative GN: Increased numbers of cells within the glomerulus due to proliferation of cells of the glomeruli and/or infiltration of polymorphs and macrophages.

- These are **immune complexes mediated** diseases. The **inciting antigen** may be:
 - **Exogenous:** For example, postinfectious glomerulonephritis, which commonly follows streptococcal infection.
 - **Endogenous:** For example, systemic lupus erythematosus (SLE).
- Characterized histologically by **diffuse proliferation of glomerular cells, and infiltration by leukocytes**.

POSTSTREPTOCOCCAL (POSTINFECTIOUS) GLOMERULONEPHRITIS

Acute poststreptococcal glomerulonephritis: Develops after streptococcal infection in children and young adults.

Poststreptococcal glomerulonephritis is a common disorder in developing countries.

Age group: Most frequent in **children between 6 to 10 years** of age, but may develop in adults.

Etiology and Pathogenesis

Q. Describe the etiopathogenesis of acute diffuse proliferative glomerulonephritis/poststreptococcal glomerulonephritis.

- It follows **streptococcal infection** (hence post-streptococcal) rather than direct primary infection of the kidney by streptococci.
- **Primary streptococcal infection** usually involves the **pharynx** (pharyngitis) or the skin (impetigo/pyoderma). Skin infections are usually associated with overcrowding and poor hygiene.
- Only **certain strains of group A β -hemolytic streptococci** are **nephritogenic**. More than 90% are due to **types 12, 4, and 1**. These can be identified by typing of M protein of the streptococcal cell wall. Main streptococcal antigenic component responsible for immune reaction in poststreptococcal glomerulonephritis is streptococcal pyogenic exotoxin B (SpeB) in most but not all cases.
- Manifests usually after a **latent period of 1 to 4 weeks** following primary streptococcal infection.
- It is an **immunologically mediated** disease.

Evidences to Support Immunological Basis

Poststreptococcal glomerulonephritis: Immune-complex mediated disease.

- **Latent period:** It ranges from 1–4 weeks, between the streptococcal infection and beginning of glomerulone-

phritis and is compatible with the **time required for the production of antibodies and the immune complex formation.**

- **Antibodies against streptococcal antigens:** They are increased in majority of patients. These antibodies includes:
 - Anti-streptolysin O
 - Anti-deoxyribonuclease B (anti-DNase B)
 - Anti-streptokinase
 - Anti-hyaluronidase
 - Anti-nicotinyl adenine dinucleotidase.
- **Hypocomplementemia:** It is observed in **more than 90% of patients**, due to activation and utilization of complement components by immune complexes.
- **Immune-complex deposits:**
 - Electron microscopy shows glomeruli with **electron dense deposits** of immune complexes.
 - Immunofluorescence shows **granular fluorescence** to the immune deposits.
- **Streptococcal antigens in the glomeruli:** Many cationic antigens unique to nephritogenic strains of streptococci, can be demonstrated in the glomeruli. Examples:
 - Nephritis-associated streptococcal plasmin receptor (NAPIR).
 - Streptococcal pyogenic exotoxin B (**SpeB**) (main antigenic determinant responsible for immune reaction in most cases of poststreptococcal glomerulonephritis) and its zymogen precursor (**zSpeB**).
 - Streptococcal GAPDH.

Mechanism of Damage

- Immune complexes are **formed in the circulation** and gets **deposited within glomeruli.**
- These immune complexes **initiate inflammation** by **activating complement** and other humoral and **cellular mediators of inflammation** (refer pages 124-7).
- The inflammatory mediators attract and **activate neutrophils and monocytes** and stimulate proliferation of mesangial and endothelial cell. The result is hypercellular glomerulus.

MORPHOLOGY

Q. Describe the morphology of kidney in acute diffuse proliferative glomerulonephritis.

Gross

The kidneys are enlarged and show pale capsular surface and cortex.

Microscopy (Fig. 21.7)

Poststreptococcal glomerulonephritis:

- Diffuse cellular proliferation of endothelial and mesangial cells
- Infiltration by leukocytes.

1. Glomeruli:

- **Enlarged hypercellular glomerulus:** It is the **classical diagnostic feature**, which is **seen in all glomeruli** (hence called **diffuse**). The hypercellularity is **due to**:
 - **Infiltration by leukocytes:** It includes both **neutrophils and monocytes**.
 - **Proliferation and swelling of endothelial and mesangial cells.**
 - **Rarely proliferation of parietal cells** lining Bowman's capsule: It forms **crescent**. Presence of crescents is a **poor prognostic feature**.
- **Obliteration of glomerular capillary lumen:** It is due to **swelling and proliferation of endothelial and mesangial cells + infiltration by leukocytes**.

2. **Tubules:** It may contain **red cell casts** in the lumen and the tubular epithelial cells may show degenerative changes.

3. **Interstitium:** It may show edema and inflammatory cell infiltrate.

4. **Blood vessels:** Unremarkable.

Immunofluorescence Microscopy (Fig. 21.8)

Poststreptococcal glomerulonephritis: Granular immunofluorescence.

Granular deposits of IgG, IgM, and C3 in the mesangium and along the GBM → **granular fluorescence**.

Electron Microscopic Findings (Fig. 21.9)

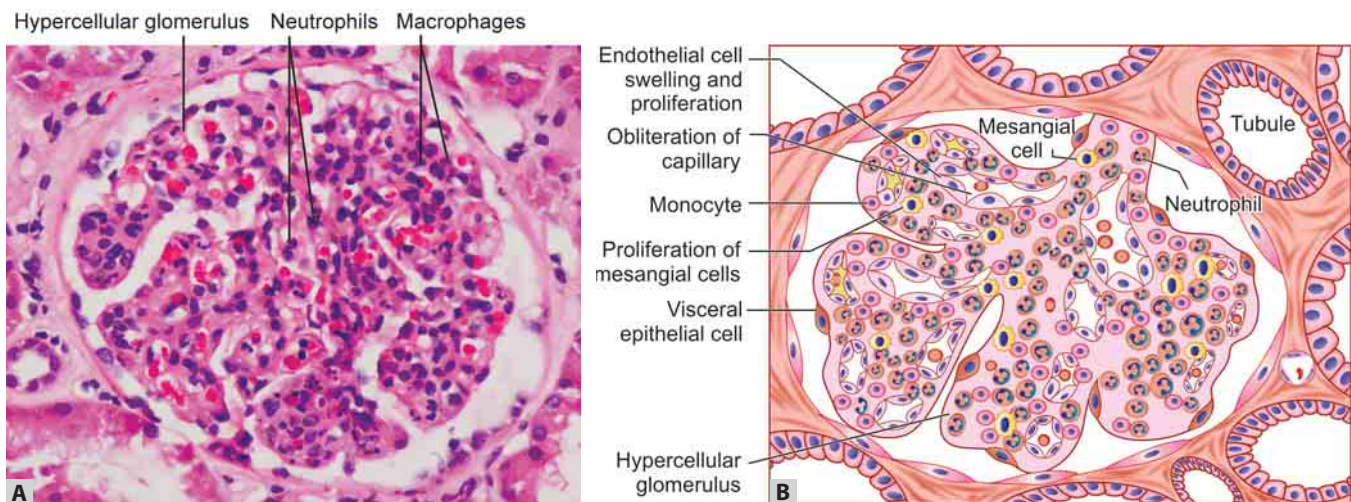
Poststreptococcal glomerulonephritis: Subepithelial deposits (humps) on EM.

- **Subepithelial deposits of discrete, amorphous, electron-dense deposits** is **characteristic feature**. These appear as dome-shaped "humps" → termed as '**humps**' or '**lumpy**' deposits. They correspond to the granular IgG and C3 demonstrated by the immunofluorescence.
- Subendothelial, intramembranous and mesangial deposits may also be present.

Clinical Course

Acute proliferative glomerulonephritis:

- Periorbital edema
- Mild to moderate hypertension
- Cola-colored urine.
- **Affected child develops malaise, fever, nausea, oliguria, and hematuria (smoky or cola-colored urine) 1 to 2 weeks after recovery from a sore throat.**



Figs 21.7A and B: Light microscopic appearance of acute poststreptococcal glomerulonephritis. (A) (Hematoxylin and Eosin stain) and (B) (diagrammatic). Glomerulus is hypercellular is due to proliferation of intrinsic glomerular cells and infiltration by leukocytes

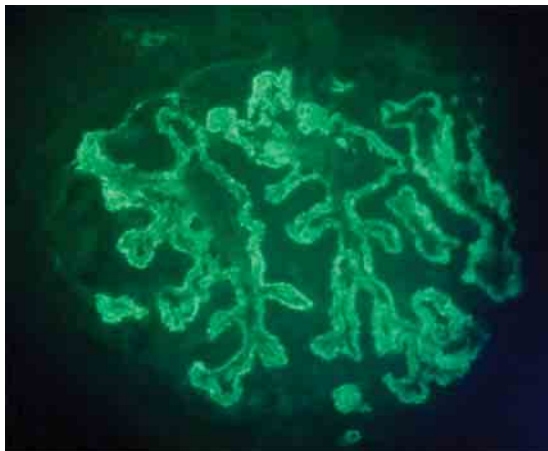


Fig. 21.8: Granular immunofluorescence characteristic of circulating or in situ immune complex nephritis

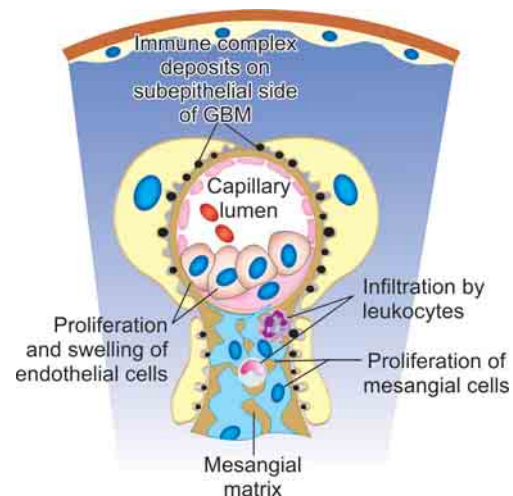


Fig. 21.9: Diagrammatic electron microscopic appearance of poststreptococcal glomerulonephritis. Subepithelial immune complexes appear as characteristic hump-like structures. Other features are endothelial and mesangial cell proliferation with narrowing of the lumen

- **Periorbital edema**, and **mild to moderate hypertension** is usually observed.
- In adults, clinical features are atypical.

Pitting edema: Does not distinguish nephritic from nephrotic syndrome.

Laboratory Findings

Q. Laboratory findings in nephritic syndrome.

Blood Findings

- **Raised antistreptococcal antibody titers:** e.g. anti-streptolysin O (ASO) titer), but the level may be low in GN that follows streptococcal skin infection.

- **Hypocomplementemia:** Significant **reduction in serum concentration of complement (C3) components.**
- Raised blood urea and serum creatinine indicate renal impairment.

Complement levels in poststreptococcal GN:

- Transient reduction during the disease
- Return to normal in 6–8 weeks.

Urinary Findings

- **Oliguria**
- **Mild** (variable degree) **proteinuria** (usually less than 1 g/day).
- **Hematuria** (smoky or cola-colored urine).

- **Red cell casts or dysmorphic red cells (red cells with cellular protrusions or fragmentation).**

Hematuria with dysmorphic RBCs are seen in: Acute glomerulonephritis.

Prognosis

Poststreptococcal glomerulonephritis: Most affected children recover; in adults prognosis is worse.

- **Children:** Prognosis is **good** in children and more than 95% totally recover.
- **Adults:** In adults, it is **less benign**. They may recover promptly or develop rapidly progressive glomerulonephritis or progress to chronic glomerulonephritis.

Nonstreptococcal Acute Glomerulonephritis (Postinfectious Glomerulonephritis)

Acute proliferative glomerulonephritis may also occur in association with other infections:

- **Bacteria:** Examples, staphylococcal endocarditis, pneumococcal pneumonia, and meningococcemia.
- **Virus:** Examples, hepatitis (B and C), mumps, HIV infection, varicella, and infectious mononucleosis.
- **Parasite:** Examples, malaria, toxoplasmosis.

The morphological changes are similar to poststreptococcal glomerulonephritis.

RAPIDLY PROGRESSIVE (CRESCENTIC) GLOMERULONEPHRITIS

Q. Write short note on rapidly progressive glomerulonephritis.

Definition: Rapidly progressive glomerulonephritis (RPGN) is a syndrome, characterized by **rapid and progressive loss of renal function** associated with **severe oliguria** and signs of **nephritic syndrome**. If not treated, death occurs due to renal failure within weeks to months.

- RPGN does not represent a specific etiologic type of glomerulonephritis. Most common microscopic features is the presence of crescents in most of the glomeruli. hence the term crescentic glomerulonephritis.

RPGN: Clinical entity with features of nephritic syndrome and rapid loss of renal function.

Classification

RPGN classified into three types based on immunological findings (Box 21.4). Each type may be idiopathic or associated with a known disorder.

Type I (Anti-GBM Antibody)

- Type I RPGN is an uncommon, aggressive type **caused by an anti-GBM antibody**. One of the examples is Goodpasture syndrome.

Type II (Immune Complex)

- It is due to **immune complex deposition** and may complicate of any of the immune complex nephritides (e.g. postinfectious glomerulonephritis, lupus nephritis).
- **Immunofluorescence:** Shows **granular fluorescence** characteristic of immune complex nephritis.

Type III (Pauci-immune)

- It is also known as pauci-immune type.
- It **neither shows anti-GBM antibody nor immune complex** by immunofluorescence and electron microscopy.
- Majorities are **idiopathic** and have circulating **antineutrophil cytoplasmic antibodies (ANCA)**.
- Some may a component of a systemic vasculitis, such as Wegener granulomatosis.

Pathogenesis

The RPGN is a syndrome caused by **primary glomerular diseases as well as systemic diseases**. Pathogenetic

BOX 21.4: Classification of rapidly progressive glomerulonephritides

Type I (Anti-GBM antibody)

Goodpasture syndrome

Type II (Immune complex)

Idiopathic

Postinfectious glomerulonephritis

Lupus nephritis

IgA nephropathy

Type III (Pauci-immune)

Idiopathic

Wegener granulomatosis

ANCA-associated

Abbreviations: GBM, glomerular basement membrane; ANCA, antineutrophil cytoplasmic antibodies.

mechanism varies but most are immunologically mediated and it indicates severe glomerular damage.

GOODPASTURE SYNDROME

Goodpasture syndrome: Anti-GBM disease in which anti-GBM antibodies cross-react with alveolar basement membrane of lung.

In Goodpasture syndrome, **anti-GBM antibodies cross-react with pulmonary alveolar basement membranes** causing pulmonary hemorrhage associated with acute renal failure.

Pathogenesis

Genetic predisposition: High prevalence of certain HLA subtypes (e.g. HLA-DRB1) in these patients point towards genetic predisposition to autoimmunity.

- **Anti-GBM antibodies** are formed against **Goodpasture antigen**. This antigen results from conformational changes in the $\alpha 3$ chain of type IV collagen of GBM.
- **Anti-GBM antibodies cross react** with the **target antigen**, which is also expressed on **pulmonary alveolar capillary basement membranes**. This results in **pulmonary hemorrhages** and **hemoptysis**, which may be life-threatening.

Goodpasture antigen: A peptide within the noncollagenous portion of the $\alpha 3$ chain of collagen type IV of GBM.

MORPHOLOGY

Gross

- Kidneys are enlarged and pale.
- May show **petechial hemorrhages on the cortical surfaces** (flea bitten kidney).

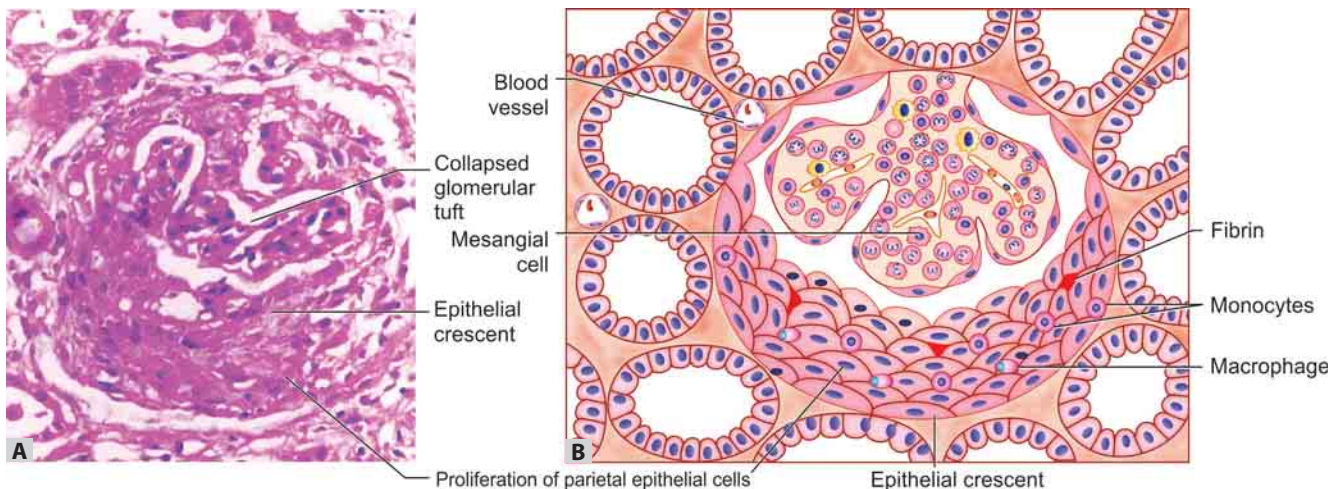
Microscopy (Fig. 21.10)

- **Glomeruli:** Its characteristic feature is **crescents** in most of the glomeruli. Hence, the term crescentic glomerulonephritis.
- **Crescents**
 - **Components:** Crescents are formed by:
 - ♦ **Proliferation of the parietal epithelial cells** lining Bowman capsule.
 - ♦ Infiltration of **monocytes and macrophages** into the urinary space.
 - **Stimulus:** **Fibrin** leaked into the Bowman space is the stimulus and is found between the cellular layers. Fibrin is derived from fibrinogen, which escaped through the **ruptured GBM** into Bowman space.
 - **Significance:** It indicates **severe glomerular injury** and does not represent any specific etiological factor.

Epithelial crescents: Proliferation of parietal epithelial cells + monocytes and macrophages.

Crescents: Formed due to fibrin leaked through ruptured GBM.

- **Tubules:** The tubular lumen may show casts and red cells.
- **Interstitium:** It shows edema with few inflammatory cells.
- **Blood vessels:** Normal.



RPGN (crescentic GN): Prognosis depends upon number of crescents as it correlates with oliguria.

Figs 21.10A and B: (A) Hematoxylin and Eosin stain (H & E stain); (B) Diagrammatic representation of crescentic glomerulonephritis showing crescent-shaped mass of proliferating parietal epithelial cells and leukocytes internal to Bowman capsule

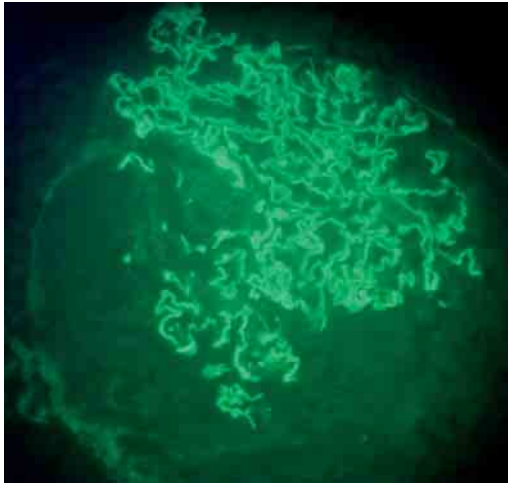


Fig. 21.11: Linear immunofluorescence for immunoglobulin G is seen along the GBM in Goodpasture syndrome

Immunofluorescence Microscopy

- Goodpasture syndrome show **linear GBM fluorescence** (Fig. 21.11) for IgG and C3 complement.

Electron Microscopy

- **Ruptures in the GBM:** It allows **leukocytes, proteins, and inflammatory mediators to leak** into the urinary space and initiates crescent formation (Fig. 21.12).

Clinical Course

In Goodpasture syndrome patient may present with **recurrent hemoptysis** or life-threatening pulmonary hemorrhage. Other features are variable hypertension and edema.

Uremia is the cause of death in Goodpasture syndrome.

Urine findings: 1) Hematuria, 2) RBC casts, and 3) moderate proteinuria.

Prognosis: Milder forms may recover, but the renal involvement usually progress within weeks and cause severe oliguria.

Goodpasture syndrome:

1. Anti-GBM disease
2. Epithelial crescent
3. Linear immunofluorescence.

NEPHROTIC SYNDROME

Q. Define nephrotic syndrome.

Nephrotic syndrome:

- Massive proteinuria
- Edema
- Lipiduria.
- Hypoalbuminemia
- Hyperlipidemia

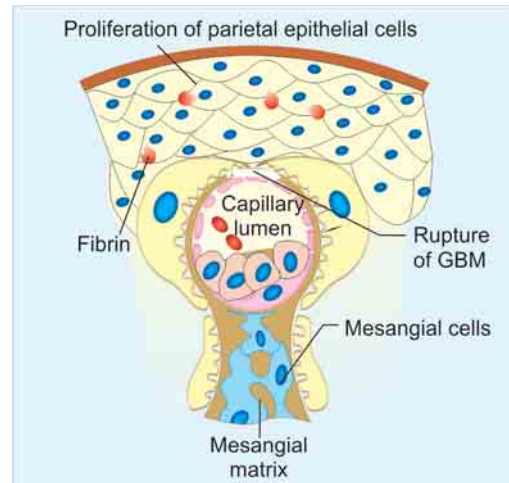


Fig. 21.12: Diagrammatic electron microscopic appearance of crescentic (rapidly progressive) glomerulonephritis. Shows disruption of GBM and proliferation of parietal epithelial cells to form crescent

Definition: Nephrotic syndrome is characterized by:

- **Massive/heavy proteinuria** (>3.5 g of protein/24 hours)
- **Hypoalbuminemia**
- **Edema**
- **Hyperlipidemia and lipiduria.**

Pathophysiology (Fig. 21.13)

In nephrotic syndrome damage to the glomerular capillary walls causes increased permeability to plasma proteins.

Nephrotic syndrome: Massive proteinuria >3.5 g/24 hours and fatty casts in urine.

1. **Massive proteinuria:** It is characterized by **daily loss of 3.5 g or more of protein** (less in children) in the **urine**.
 - Normally, the glomerular capillary wall acts as a size and charge dependent barrier for the plasma filtrate.
 - Proteinuria is **due to increased permeability of glomerular capillary wall to plasma proteins**.
 - The major proportion of protein lost in the urine is **albumin (low-molecular-weight proteins)**, and rarely globulins (high-molecular-weight proteins). The ratio between low and high-molecular-weight proteins in the urine in various cases of nephrotic syndrome is due to selectivity of proteinuria. Types of glomerular proteinuria are presented in Box 21.5.

Albumin is the major protein lost in the urine, but globulins may also be excreted in some diseases.

Q. Write short note on selective and non-selective proteinuria.

Albumin is negatively charged and repelled by negatively charged normal GBM.

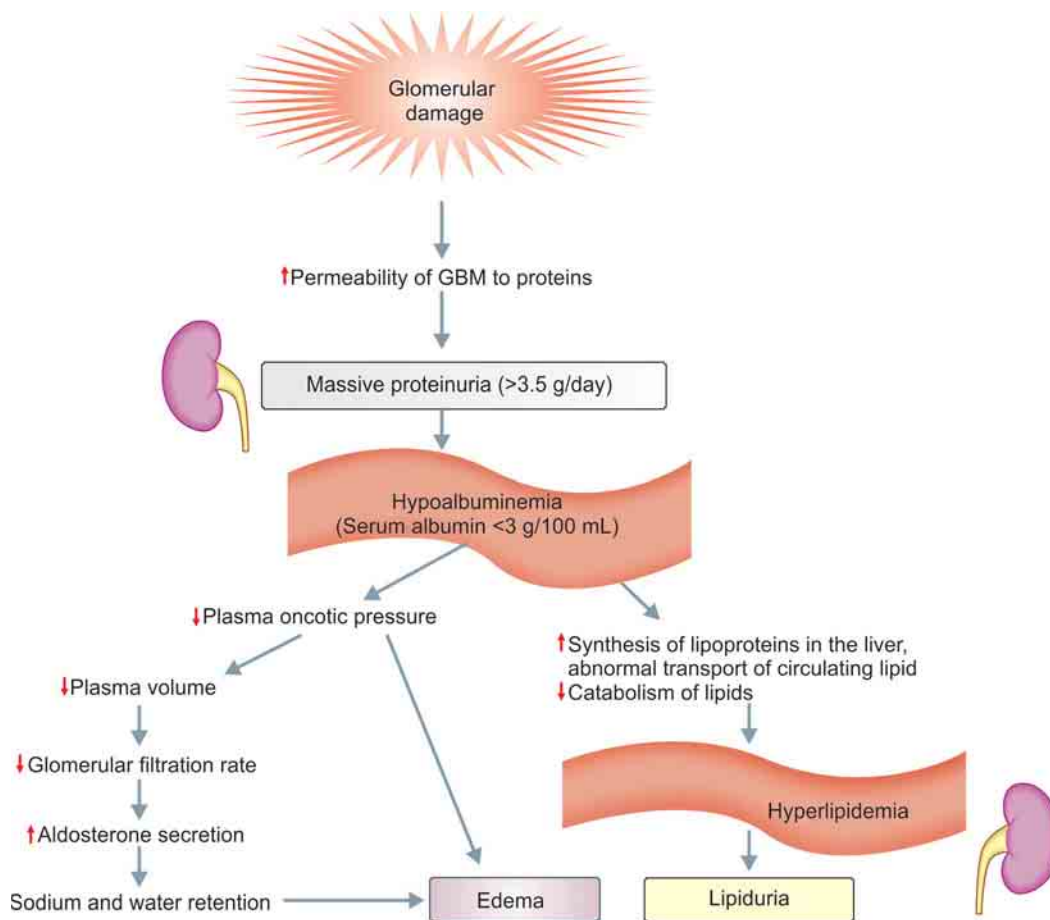


Fig. 21.13: Various characteristic features of nephrotic syndrome and its mechanism
Abbreviation: GBM, glomerular basement membrane.

NPHS1 gene encodes: Nephrin and its mutations cause congenital nephrotic syndrome.

NPHS2 gene encodes: Podocin and its mutations cause acquired steroid resistant nephrotic syndrome.

Nephrotic syndrome: Increased the susceptibility to

- Coronary artery disease
- Infections
- Thrombosis and embolism.

BOX 21.5: Types of glomerular proteinuria

- **Highly selective proteinuria:** In this type, only low-molecular weight/intermediate-sized (<100kDa) proteins (such as albumin 70 kD, transferrin 76 kD molecular weight) leaks through the glomerulus, e.g. minimal-change disease
- **Non-selective (poorly-selective) proteinuria:** It is characterized by leakage of range of different proteins (proteins of any size) including larger/higher molecular-weight serum proteins (e.g. immunoglobulins) in addition to albumin through the glomerulus, e.g. membranous nephropathy

In renal disease, albumin is first to appear in urine because it has molecular weight slightly greater than the molecules normally getting filtered.

2. **Hypoalbuminemia:** Plasma albumin levels less than 3 g/dL.
 - Massive proteinuria decreases the serum albumin levels (hypoalbuminemia).
 - Hypoalbuminemia decreases the colloid osmotic pressure of the blood.

3. Generalized edema:

- **Causes:**
 - **Decreased colloid osmotic pressure of the blood:** It leads to accumulation of fluid in the interstitial tissues → generalized edema.
 - **Sodium and water retention:** Decreased plasma volume reduces glomerular filtration rate which in turn leads to **compensatory secretion of aldosterone**. It aggravates the edema by salt and water retention.
- **Characteristics of edema:**
 - Soft and pitting
 - Most marked in the **periorbital regions** and **dependent portions of the body**
 - When massive associated with pleural effusions and ascites.

4. Hyperlipidemia and lipiduria:

Hyperlipidemia:

- Most patients with nephrotic syndrome have raised blood levels of cholesterol, triglyceride, very-low-

density lipoprotein, low-density lipoprotein, Lp(a) lipoprotein, and apoprotein.

- **Causes:** Hyperlipidemia due to:
 - Increased synthesis of lipoproteins in the liver
 - Abnormal transport of circulating lipid
 - Decreased catabolism of lipids.

Lipiduria:

- Hyperlipidemia is followed by **leakage of lipoproteins** across the glomerular capillary wall → leaked lipoprotein is reabsorbed by tubular epithelial cells → then shed along with the degenerated cells → appears **in urine either as free fat or as oval fat bodies**.

Major Complications of Nephrotic Syndrome

- **Coronary atherosclerosis:** Hyperlipidemia predisposes to atherosclerosis of coronary artery.
- **Infections:** Nephrotic patients are **susceptibility to infection**, especially staphylococci and pneumococci. It may be **due to loss of immunoglobulins in the urine**.
- **Thrombotic and thromboembolic complications:** This may be due to loss of endogenous anticoagulants (e.g. antithrombin III) and antiplasmins in the urine.

Causes of Nephrotic Syndrome (Table 21.2)

Q. Write short note on causes of nephrotic syndrome.

- Most common systemic causes of the nephrotic syndrome are **diabetes, amyloidosis, and SLE**.

TABLE 21.2: Causes of nephrotic syndrome

Primary glomerular diseases	Systemic diseases
Membranous glomerulopathy (~30% in adults)	Diabetes mellitus
Minimal-change disease (~75% in children)	Amyloidosis
Focal segmental glomerulosclerosis (~35% in adults)	Infections (malaria, syphilis, hepatitis B and C, HIV)
Membranoproliferative glomerulonephritis	Systemic lupus erythematosus
IgA nephropathy	Drugs (penicillamine, "street heroin")
	Malignancy (carcinoma, melanoma)
	Others (bee-sting allergy, hereditary nephritis)

Nephrotic syndrome: Glomerular inflammation less than in nephritic syndrome.

- Most important primary glomerular lesions causing nephrotic syndrome are **minimal-change disease, membranous glomerulopathy, and focal segmental glomerulosclerosis**.

MEMBRANOUS NEPHROPATHY (MEMBRANOUS GLOMERULOPATHY)

Q. Write short note membranous glomerulopathy.

Membranous nephropathy: Common primary cause of the nephrotic syndrome in adults.

Membranous nephropathy is characterized by **uniform diffuse thickening of the glomerular capillary wall**. This is due to the accumulation of **electron-dense deposits along the subepithelial side of the glomerular basement membrane**.

Membranous nephropathy is the **common cause (~30%) of the nephrotic syndrome in adults**.

Etiology

- **Primary/Idiopathic:** No identifiable cause in about 85% of patients.
- **Secondary:** Found in association with:
 - **Therapeutic drugs:** For example, penicillamine, captopril, nonsteroidal anti-inflammatory drugs (NSAIDs), gold
 - **Malignant neoplasms:** For example, carcinomas of the lung and colon, lymphomas and melanoma
 - **Autoimmune disease:** For example, SLE, Hashimoto thyroiditis
 - **Infections:** For example, chronic hepatitis B, hepatitis C, malaria, syphilis, schistosomiasis.

Pathogenesis

Membranous glomerulopathy is an **chronic immune complex-mediated disease**:

- **Idiopathic:** It is an **autoimmune disease** caused by antibodies reacting to an endogenous or planted glomerular antigen. Autoimmune reaction by auto-antibodies are most often directed against phospholipase A2 receptor antigen on the podocyte. It results in **situ formation of immune complex**. Heymann nephritis is one of the experimental model of membranous nephropathy/glomerulopathy.

Membranous nephropathy: Autoantibodies are directed against phospholipase A2 receptor on the podocyte.

- **Secondary:** It results from **circulating immune complex** and the antigens may be:
 - **Exogenous antigens:** For example, hepatitis B, *Treponema*.

- **Endogenous antigens:** For example, nonrenal antigens (e.g. thyroglobulin) or renal antigens.

Mechanism of Glomerular Injury

- **Glomeruli do not show any inflammatory cells** (like neutrophils, monocytes), or platelets.
- **Glomeruli show complement that indicates direct action of C5b-C9** (membrane attack complex).
- **The C5b-C9 activates glomerular epithelial and mesangial cells** → **liberates proteases and oxidants** → **cause capillary wall injury** → leakage of protein.

MORPHOLOGY

Membranous glomerulopathy: Nephrotic syndrome, diffuse GBM thickening due to expansion of the basement membrane.

Light Microscopy (Fig. 21.14)

1. **Glomeruli:**
 - Show **uniform, diffuse thickening of the glomerular capillary wall**.
 - **Normocellular glomeruli:** There is **neither proliferation nor inflammation**.
 - **Special stains:** **Silver stains (black color)** or **periodic acid-Schiff stain (pink color)** show **characteristic spikes** in all glomeruli, **between the deposits of immune complex**.
 - As the disease progresses glomeruli may become totally sclerosed.
2. **Tubules:** The **epithelial cells of the proximal tubules** show **droplets of reabsorbed protein**. Later tubules undergo atrophy.
3. **Interstitial:** It shows **mononuclear cell inflammatory cells** and **interstitial fibrosis** in late stages.
4. **Blood vessels:** Early stages vessels appear normal.

Membranous nephropathy: Uniform GBM thickening, normocellular glomerulus with little or no inflammation.

Electron Microscopy (Fig. 21.15)

- **Thickening of the glomerular capillary wall** due to **irregular electron-dense deposits of immune complexes** between the basement membrane and the overlying visceral epithelial cells (**subepithelial**). The **visceral epithelial cells** show **effaced foot processes**.
- **In between these immune deposits, basement membrane material is laid down**, which appear as **irregular spikes protruding from the GBM**.

Membranous glomerulopathy: Characteristic spikes between the deposits of immune complex better appreciated in PAS or silver stains.

Changes due to subepithelial immune complexes are divided into stages:

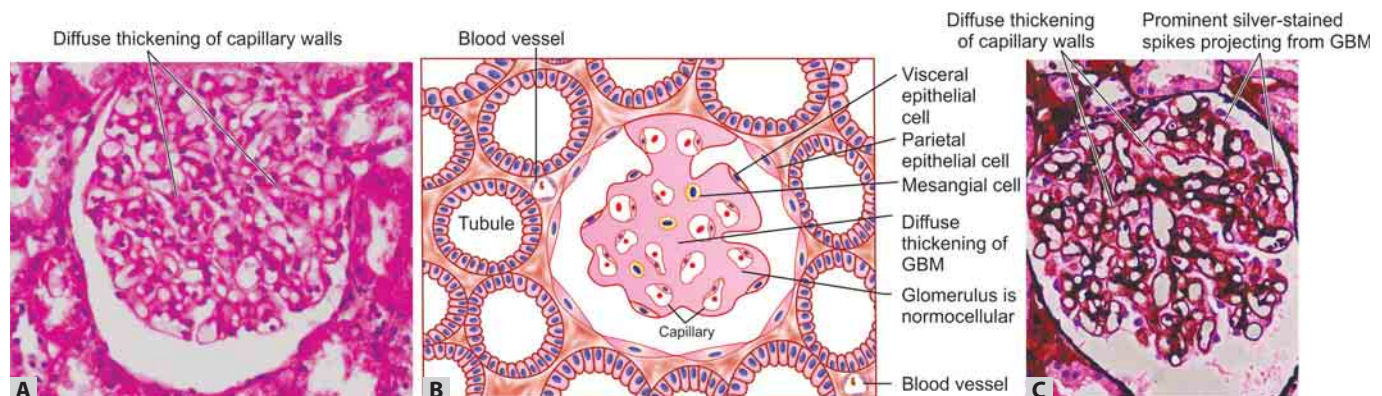
- **Stage I:** Capillary walls are normal or show mild thickening and subepithelial dense deposits.
- **Stage II:** Projections of GBM material around dense deposits, which appear as spikes on the epithelial surface of the basement membrane, which stain with silver.
- **Stage III:** New basement membrane encircles the deposits and is laid over the immune deposits, which bury them within a markedly thickened, irregular membrane. In silver stain, they appear dome-like protrusions.
- **Stage IV:** The immune deposits undergo degradation and lysis.

Immunofluorescence Microscopy

Membranous nephropathy:

- Granular IF
- Subepithelial deposits on EM.

It shows **granular deposits** of both **immunoglobulins and complement (IgG and C3)** in the **subepithelial** (between the visceral epithelial cell and the GBM) region. There is also intense staining for membrane attack complex.



Figs 21.14A to C: Light microscopy (A) Hematoxylin and Eosin stain (H & E); (B) Diagrammatic of membranous nephropathy showing marked diffuse thickening of the GBM without an increase in the number of cells; (C) Silver methenamine stain showing marked thickening of capillary walls and prominent silver-stained spikes projecting from GBM

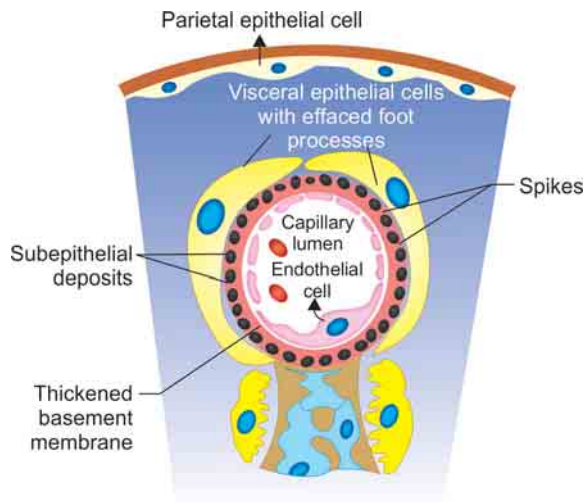


Fig. 21.15: Diagrammatic representation of electron microscopy of membranous glomerulopathy. Electron-dense deposits are seen along the epithelial side of the basement membrane. The visceral epithelial cells show effacement of foot processes overlying deposits

Clinical Features

- Present with **nephrotic syndrome**
- **Nonselective proteinuria** (which is selective in minimal-change disease).
- **Does not respond to corticosteroid therapy.**

Course: Variable but generally indolent.

MINIMAL-CHANGE DISEASE

MCD: Common cause of nephrotic syndrome in children.

Minimal-change disease (MCD) is named so because the **glomerular changes are absent or minimal and glomeruli appear normal under light microscopy**. But under electron microscopy, it shows **diffuse effacement (loss) of foot processes of visceral epithelial cells (podocytes)**.

MCD is the **major cause of nephrotic syndrome in children**, but it is less common in adults. MCD is relatively benign disorder.

Age: Peak incidence between 2 and 6 years of age.

MCD: Historical original name was lipoid nephrosis, because the most noticeable feature by light microscopy was fat in the renal tubular epithelial cells.

Etiology and Pathogenesis

Q. Write short note on etiology and pathogenesis of minimal-change disease.

The pathogenesis of MCD is unknown. The electron microscopic changes indicate that **primary injury is to the visceral epithelial cells**, which may be caused by either immunological or nonimmunological mechanism.

1. **Immunological mechanism:** Lack of immune deposits in the glomerulus that excludes the possibility of immune complex mechanism. However, several features suggest immunological mechanism:

- Sometimes **follows a respiratory infection or routine prophylactic immunization.**
- Dramatically **responds to corticosteroids** and/or other **immunosuppressive therapy.**
- **Associated with atopic/allergic disorders** (e.g. eczema, rhinitis).
- **Increased incidence in patients with Hodgkin lymphoma**, which has defects in T-cell-mediated immunity.

Steroid resistant nephrotic syndrome: Mutation in the gene encoding podocin.

- Increased prevalence of certain **HLA haplotypes** suggesting a genetic predisposition.
- Immune dysfunction causes production of a **cytokine** by T-cells. The cytokine **damages visceral epithelial cells** and increases glomerular permeability causing **proteinuria**.

Finnish type of congenital nephrotic syndrome: Mutation in the nephrin gene.

2. **Nonimmunological mechanism:** It may cause injury to visceral epithelial cell.

- **Mutations** in structural proteins namely **nephrin and podocin** (localized to the slit diaphragm) causes structural defects of the podocyte leading to marked proteinuria.
- **Mutation in the nephrin gene** is found in hereditary type of **congenital nephrotic syndrome (Finnish type)** with minimal-change glomerular morphology.

Mechanism of Proteinuria

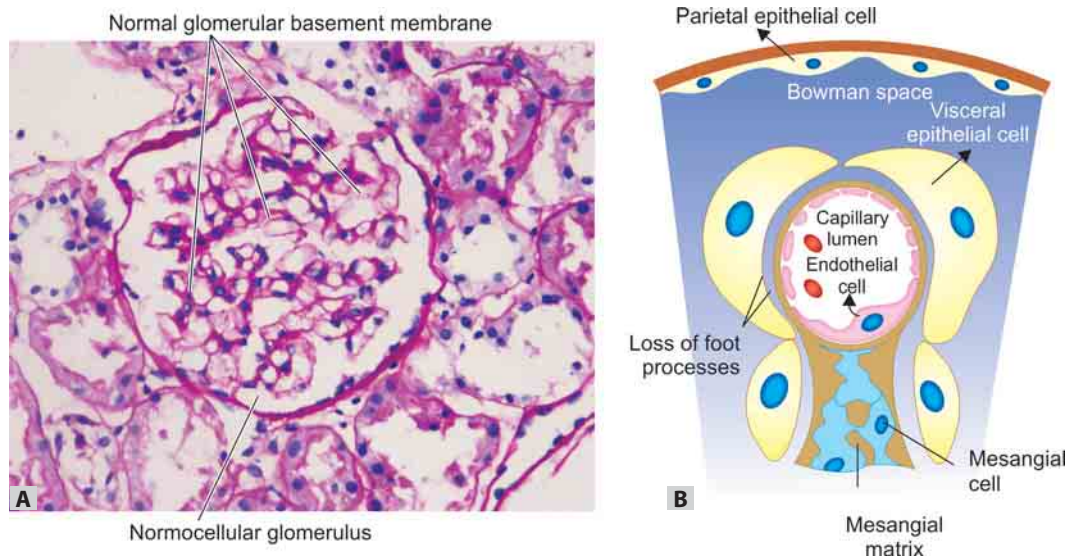
- MCD is accompanied by a **loss of glomerular polyanions** on the GBM.
- **Defects in the charge barrier** allow anionic proteins, particularly **albumin**, to pass more easily through the GBM → **massive proteinuria**.

MORPHOLOGY

Q. Write short note on morphology of minimal-change disease.

Gross

- Kidneys are mildly enlarged with smooth capsular surface.
- Cut section shows pale to yellow cortex.



Figs 21.16A and B: Minimal-change disease: (A) Light microscopy. PAS stain shows normal GBM without any cellular proliferation; (B) Diagrammatic electron microscopic appearance. It shows effacement of the foot processes of visceral epithelial cells. All other glomerular structures appear normal

Light Microscopy

MCD:

- Glomeruli appear normal by light microscopy
- Proximal tubules contain lipid vacuoles in the cytoplasm.
- **Glomeruli:** Appear normal by light microscopy.
- **Tubules:** The cytoplasm of epithelial cells lining the proximal tubules contains lipid vacuoles and glassy (hyaline) protein droplets (hence, the historical term lipid nephrosis for this disease). These are due to the tubular reabsorption of lipoproteins passing through damaged glomeruli.
- **Interstitium:** May show mild edema.
- **Blood vessels:** Appear normal.

Electron Microscopy (Fig. 21.16)

MCD: EM shows uniform and diffuse effacement/loss of foot processes in the visceral epithelial cells.

- GBM appears normal without any electron-dense deposit.
- **Changes in the visceral epithelial cells:** Most characteristic feature is uniform and diffuse effacement of foot processes in the visceral epithelial cells. This change is wrongly called as “fusion” of foot processes. The cytoplasm shows vacuolization and swelling.

Immunofluorescence

Does not show immunoglobulin or complement deposits.

Clinical Features

- Classically, it presents as **nephrotic syndrome**.
- Onset of nephrotic syndrome may be preceded by upper respiratory tract infection or immunization.

- **Proteinuria** usually is **highly selective** (albumin more than globulins).
- Other features: Generalized edema, hypoalbuminemia, and hyperlipidemia.
- In spite of massive proteinuria, renal function remains good.

Prognosis: Excellent.

FOCAL SEGMENTAL GLOMERULOSCLEROSIS

Focal segmental glomerulosclerosis (FSGS) is characterized by the **sclerosis that involves only part of the capillary tuft** (i.e. segmental) **of some glomeruli** (i.e. focal).

FSGS: Segmental and focal hyalinosis and sclerosis.

Classification

- **Primary (idiopathic).**
- **Secondary:**
 - Viruses: For example, HIV infection
 - Drugs: For example, Heroin addiction
 - Sickle-cell disease
 - Massive obesity
 - **Congenital** (e.g. unilateral agenesis) and acquired (e.g. reflux nephropathy) reductions in renal mass.
- **Hereditary forms:** Autosomal recessive FSGS due to mutations in the *NPHS2* gene, which maps to chromosome 1q25-q31 and encodes the protein product podocin.

Inherited mutations in genes that encode podocyte proteins, e.g. **podocin**, α -**actinin-4**. Adult-onset FSGS with mutations in the gene encoding TRPC6.

Pathogenesis

Majority of FSGS are **idiopathic**. According to some, it represents a subset minimal-change disease, because it shows similar visceral **epithelial damage**, i.e. **diffuse effacement of foot processes**.

MORPHOLOGY

Light Microscopy

The **focal and segmental lesions** involve only a minority of the glomeruli. The glomerular shows **hyalinosis and sclerosis**.

- **Hyalinosis:** It is characterized by extracellular accumulation of **homogeneous and eosinophilic material** in the glomeruli. It represents **leaked plasma proteins** due to endothelial or capillary wall injury.
- **Sclerosis:** It is an **extracellular accumulation of collagenous matrix** in the mesangium or in the capillary loops, or both. Severe hyalinosis and sclerosis → occludes the capillary lumens in affected glomerular tuft → fibrous adhesions between the sclerotic portions of glomeruli and the nearby parietal epithelium.
- Glomeruli, which do not show segmental lesions appear normal on light microscopy.

FSGS: Epithelial damage consists of degeneration and focal disruption of visceral epithelial cells with effacement of foot processes is the hallmark of FSGS.

Electron Microscopy

FSGS: Degeneration and focal disruption of the visceral epithelial cells is the hallmark feature.

Both sclerotic and nonsclerotic areas show **diffuse effacement of foot processes**.

Immunofluorescence Microscopy

IgM and C3 may be seen in the sclerotic areas and/or in the mesangium.

Collapsing glomerulopathy:

- Variant of FSGS
- Retraction and/or collapse of entire glomerular tuft
- Proliferation and hypertrophy of visceral epithelial cells
- Idiopathic or HIV associated.

Clinical Course

Focal segmental glomerulosclerosis manifest as **nephrotic syndrome or heavy proteinuria**.

Prognosis: Children have better prognosis than adults.

MEMBRANOPROLIFERATIVE GLOMERULONEPHRITIS

Membranoproliferative glomerulonephritis (MPGN) is histologically characterized by:

1. **Thickening of the glomerular basement membrane.**
2. **Proliferation of mainly mesangial cells.**
3. **Leukocyte infiltration.**

Because the proliferation is mainly mesangial cells and also involves the capillary loops, it is also known as **mesangiocapillary glomerulonephritis**.

MPGN:

- Thickening of GBM
- Proliferation of mainly mesangial cells
- Leukocyte infiltration.

MPGN: Responsible for about 10–20% of nephrotic syndrome in children and young adults.

MPGN is a histological pattern of immune-mediated injury rather than a specific disease.

Etiology

MPGN:

- Primary (two types I and II) dense deposit disease
- Secondary.
- **Idiopathic (primary):** Depending on pathogenesis, electron microscopic, and immunofluorescent appearance, idiopathic MPGN is subdivided into two major types:
 - Type I (immune complex deposit)
 - Type II now termed as dense-deposit disease and discussed in page 610-12.
- **Secondary:** MPGN can develop in association with systemic disorders.

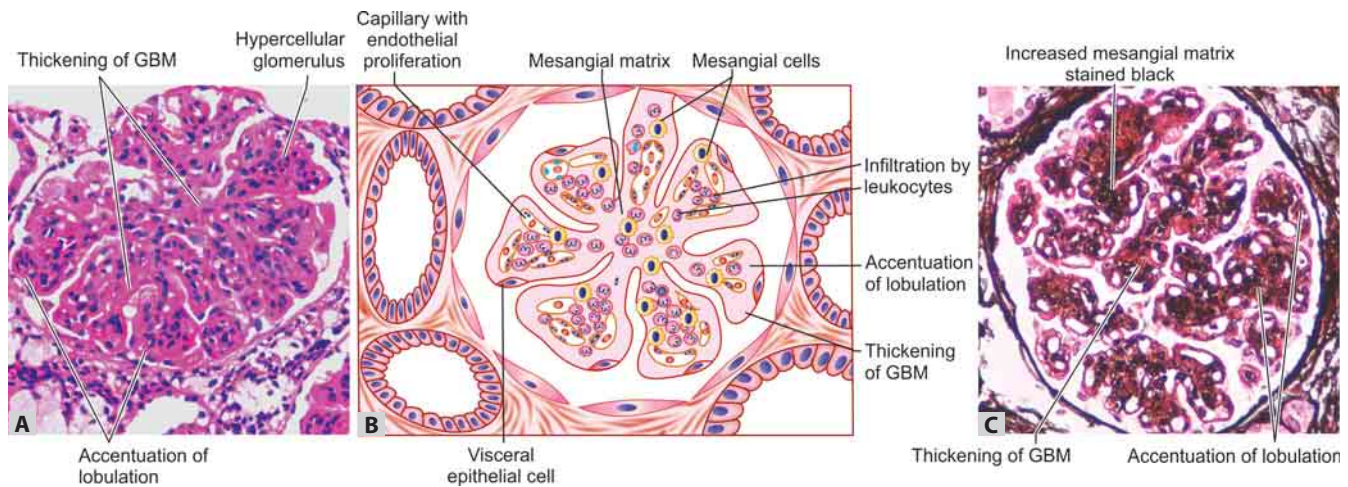
Pathogenesis of MPGN

- **Previously known as type I MPGN.** Immune complexes are deposited in the **subendothelial zone** of glomerulus.
- Origin of **nephritogenic antigen is not known**.
- It occurs due to **activation of both classical and alternative complement pathways**. It belongs to a group of disorders termed as C3 glomerulopathies.

MORPHOLOGY

Membranoproliferative GN: A combination of the thickening of basement membrane and proliferation.

- Light microscopy (Fig. 21.17)
- Both types of MPGN appear similar.



Figs 21.17A to C: Membranoproliferative glomerulonephritis: (A) Hematoxylin and eosin (H & E); (B) diagrammatic; (C) silver methenamine stain. The glomerulus shows mesangial cell proliferation, increased mesangial matrix and thickening of GBM, accentuation of lobular architecture and infiltration by leukocytes

MPGN:

- Large hypercellular glomeruli
- Accentuation of lobular architecture
- Thickening of GBM.
- **Glomeruli**
 - **Large and hypercellular glomeruli:** It is due to:
 - ◆ Proliferation of (mesangial and endothelial) cells
 - ◆ Infiltration of leukocytes
 - ◆ Epithelial crescents are seen in many cases.
 - **Accentuation of lobular architecture (lobular distortion or hypersegmentation):** It is due to the proliferating mesangial cells as well as increased mesangial matrix.

GBM: Periodic acid–Schiff (PAS) stain highlights thickening of glomerular basement membrane better than H&E.

- **Thickening of GBM:** It may be segmental and most prominent in the peripheral capillary loops.
- **Subendothelial immune complex deposits** stimulates → synthesis of new basement membrane → produces “**duplication**” of the basement membrane (commonly called as splitting) → produces “**double-contour**” or “**tram-track**” appearance due to glomerular capillary wall. This is better appreciated with **special stains such as silver or PAS**.
- Within the duplicated basement membranes **inclusion or interposition of cellular elements**, (which can be of mesangial, endothelial, or leukocytic origin) is seen. Such interposition is also **responsible for the splitting of the basement membranes**.
- **Tubules:** Epithelial cells of tubules may show vacuolation and droplets of hyaline.
- **Interstitial:** It may show mild focal chronic inflammatory cells.

- **Blood vessels:** During early stages appear normal but later may show changes due to hypertension.

Electron Microscopy and Immunofluorescence

MPGN: Subendothelial immune complex deposits, duplication of GBM → produces “double-contour” or “tram-track” appearance and nephrotic/nephritic syndrome.

- **Electron microscopy** (Fig. 21.18): Shows discrete **subendothelial electron-dense deposits**.
- **Immunofluorescence:** Its appearance suggests activation of classic complement pathway by immune complex.
 - C3 is deposited in a **granular pattern**
 - IgG and early complement components (C1q and C4) are often seen.

Clinical Features

Age: Mostly found in adolescence or young adults.

Presentation: It presents with **nephrotic syndrome**, some only with hematuria or proteinuria in the non-nephrotic range, but many have a combined **nephrotic-nephritic picture**.

Course: Slowly progressive course and about 50% develop chronic renal failure. Some may develop crescents and develop RPGN.

DENSE DEPOSIT DISEASE

Dense deposit disease (formerly called type II MPGN) in most of the patients is due to excessive activation of the alternative complement pathway (Fig. 21.19).

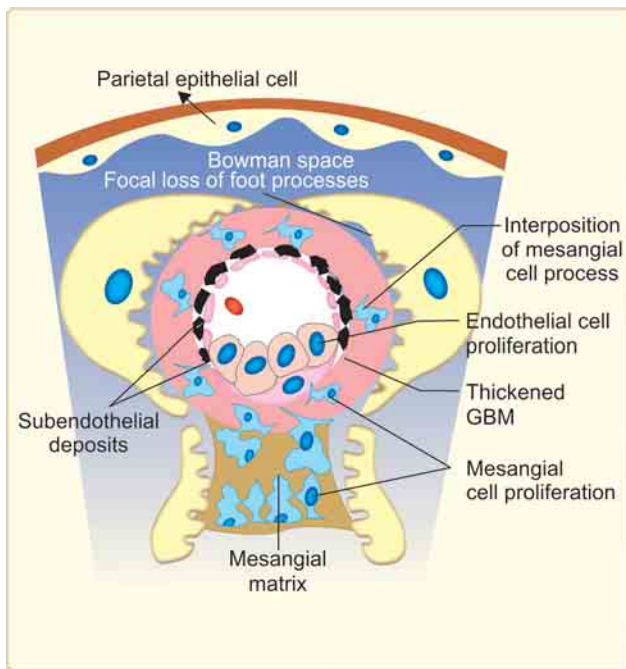


Fig. 21.18: Type 1 MPGN. Diagrammatic electron microscopic appearance of membranoproliferative glomerulonephritis characterized by hypercellular glomeruli (proliferation of endothelial and mesangial cells), thickened GBM, narrowing of the capillary lumen. Type I MPGN shows large subendothelial deposits of immune complexes, which extend along the inner border of the basement membrane

Pathogenesis

Dense deposit disease: Due to activation of alternative complement pathway.

It is due to **activation of the alternative complement pathway**, and supporting evidences for this are:

- **Decreased serum C3 but normal C1 and C4.** The early components of complement (C1 and C4) are used only in the activation of classical complement pathway.
- **Decreased serum levels of factor B and properdin**, which are the components of the alternative complement pathway.
- In the alternative complement pathway, C3 is directly cleaved to C3b. The glomeruli show deposits of C3 and properdin but not IgG.

Sequence of Events (Fig. 21.19)

- Substances like **bacterial polysaccharides, endotoxin, and aggregates of IgA** cleave C3 to C3b.
- **C3b in the presence of factors B and D** generates C3bBb (alternative pathway C3 convertase).
- **C3bBb is labile and degraded by factors I and H, but stabilized by properdin.**
- Patients with type II MPGN have an IgG autoantibody against C3 convertase in the serum known as **C3 nephritic factor (C3NeF)**.

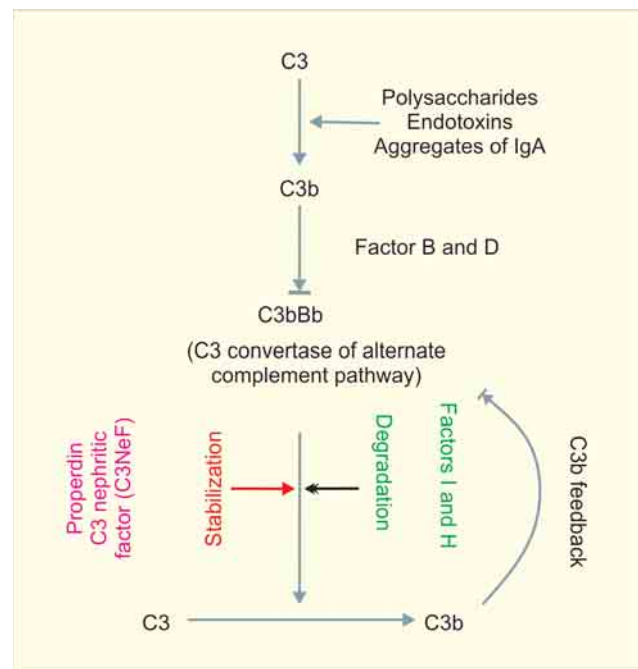


Fig. 21.19: The alternative complement pathway in MPGN. Patients of MPGN show C3NeF antibody in the serum which acts at the same step as properdin and stabilizes the alternative pathway C3 convertase. This enhances C3 activation and consumption, resulting in hypocomplementemia

- **C3NeF acts at the same step as properdin and stabilizes C3bBb and prevent its degradation.**
- This results in persistent and prolonged C3 activation and hypocomplementemia. In addition, decreased synthesis of C3 by the liver, also responsible for hypocomplementemia.

MORPHOLOGY

Light Microscopy

Dense deposit disease shows wide spectrum of microscopic changes. Some cases of them show features of MPGN described earlier.

- Majority show mainly mesangial proliferative pattern of injury, while others may show an inflammatory and focally crescentic appearance. Some cases show dense deposits of a cellular material in the glomerular basement membranes.

Electron Microscopy (Fig. 21.20)

It is characteristic and shows irregular, ribbon-like deposition of electron-dense material of unknown composition in the GBM proper (central zone named lamina dense).

Immunofluorescence

Its appearance suggests activation of alternate complement pathway.

- C3 is present in irregular granular or linear foci on either side but not within the dense deposits. C3 also seen in the mesangium forming characteristic circular aggregates termed as mesangial rings.
- IgG and early complement components (C1q and C4) are absent.

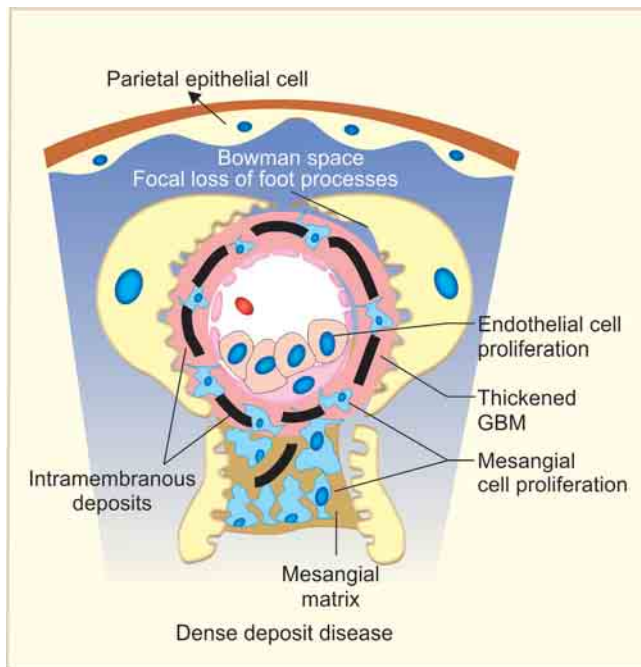


Fig. 21.20: Dense deposit diseases. Diagrammatic electron microscopic appearance of dense deposit disease (formerly called type II MPGN) intramembranous deposits

Clinical Features

- Mainly occurs in children and young adults.
- Present as nephritic syndrome with hematuria and/or nephrotic syndrome with proteinuria overlaps with that of MPGN.
- Prognosis is poor.

DIFFERENCES BETWEEN NEPHRITIC AND NEPHROTIC SYNDROME (TABLE 21.3)

Alport syndrome: EM is diagnostic and shows “basket weave appearance” of the GBM.

Alport syndrome:

- Hereditary nephritis
- Abnormal $\alpha 3$ (COL4A3), $\alpha 4$ (COL4A4), or $\alpha 5$ (COL4A5) chain of type IV collagen
- Hematuria with progression to chronic renal failure
- Nerve deafness
- Eye disorders
- Foamy cells in interstitium.

TABLE 21.3: Differences between nephritic and nephrotic syndrome

Features	Nephritic syndrome	Nephrotic syndrome
Proteinuria	1 to 2 g/day	Massive; more than 3.5 g/day
Hematuria	Present	Absent
Hypertension	Present	Absent
Hypoalbuminemia	Absent	Present; serum albumin less than 3g/dL
Hyperlipidemia and lipiduria	Not seen	Present
Edema	Mild	Moderate to severe
Azotemia and Oliguria	Present	Absent
Microscopy of urine	RBC and RBC casts	Fatty casts

CHRONIC GLOMERULONEPHRITIS

Azotemia: Elevation of blood urea nitrogen (BUN) and creatinine levels.

Q. Write short note on causes of chronic glomerulonephritis.

Chronic glomerulonephritis is an end-stage of many types of glomerulonephritis (Table 21.4).

MORPHOLOGY

Gross (Fig. 21.21):

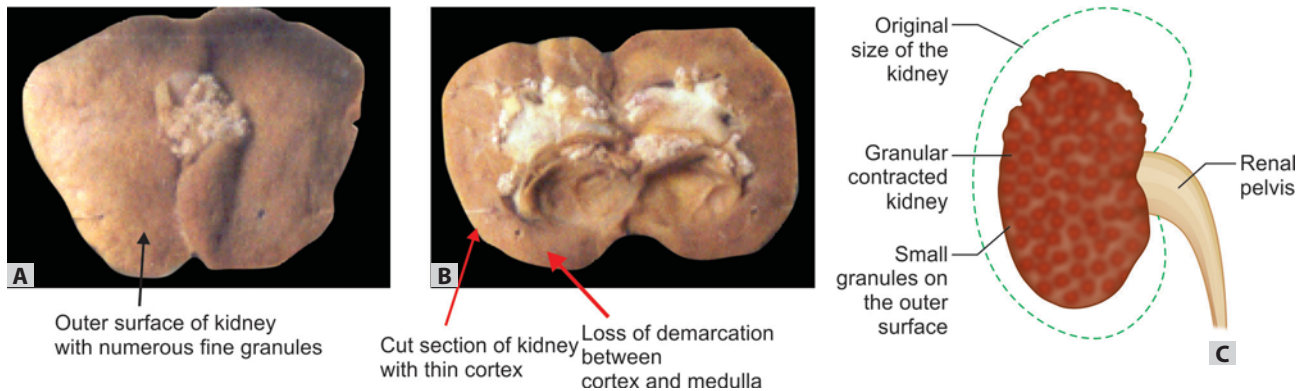
- Both kidneys show diffusely granular cortical surfaces and symmetrical contraction.
- Cut section: It shows thinned cortex and an increase in peripelvic fat. The capsule is difficult to remove because of adhesions.

Microscopy

- **Glomeruli:** The glomerular changes depend on the stage of the disease:
 - **Early stages:** It may show features of the primary disease (e.g. membranous nephropathy or MPGN).
 - **Late stages:** Obliteration of glomeruli, which appear as acellular eosinophilic masses consisting of plasma proteins, increased mesangial matrix, basement membrane-like material, and collagen.
- **Tubules:** They show marked atrophy.
- **Interstitium:** It shows fibrosis and mononuclear leukocytic infiltration.
- **Blood vessels:** Hypertension develops in patients with chronic glomerulonephritis and the vascular changes of hypertension such as arterial and arteriolar sclerosis may be seen (refer page 384-5).

TABLE 21.4: Causes of chronic glomerulonephritis

Crescentic glomerulonephritis (RPGN)	Focal segmental glomerulosclerosis (FSGS)	Non-proliferative: glomerulonephritis • Membranous glomerulonephritis • Diabetic glomerulosclerosis • Amyloidosis
Membranoproliferative glomerulonephritis (MPGN)	IgA nephropathy	
Membranous nephropathy	Poststreptococcal glomerulonephritis—rare	
Others		



Figs 21.21A to C: Gross features of chronic glomerulonephritis. (A) Outer aspect shows contracted kidney with diffusely granular surface; (B) Cut section shows thinned cortex and loss of demarcation between cortex and medulla; (C) Diagrammatic appearance of chronic GN

Uremic Complications

Patients of chronic renal failure may show complications due to uremia. These include:

- Uremic pericarditis
- Uremic gastroenteritis
- Secondary hyperparathyroidism with nephrocalcinosis and renal osteodystrophy
- Left ventricular hypertrophy due to hypertension
- Diffuse alveolar damage (uremic pneumonitis).

Clinical Course

Chronic glomerulonephritis:

- End-stage of any GN
- Granular contracted kidney
- Hyalinized glomeruli
- Progresses to renal failure.

Insidious onset: It may progress to renal insufficiency or uremia during a span of years. Most patients have hypertension and may present with hypertension induce cerebral or cardiovascular diseases.

Nonspecific complaints: These include loss of appetite, anemia, vomiting, or weakness.

It may be detected during routine medical examination or during the course of investigation of edema or urinary abnormalities.

Chronic GN: Urine shows broad waxy casts.

GLOMERULAR LESIONS ASSOCIATED WITH SYSTEMIC DISEASES

Lupus Nephritis

Q. Write short note on lupus nephritis.

- Renal (kidney) changes in SLE are known as lupus nephritis and are seen in up to 50% of SLE patients.
- It is an **immune complex mediated** (type III hypersensitivity reaction) disease.
- The main mechanism of renal injury is by immune complex, which consist of **DNA and anti-DNA antibodies**.
- Morphological features are presented in page 138.

DIABETIC NEPHROPATHY

Q. Write short note on renal changes in diabetes mellitus (diabetic nephropathy).

Diabetic nephropathy is the term used for **collective lesions that often occur together in the diabetic kidney**. Diabetic nephropathy can develop in both insulin-dependent type 1 diabetes and type 2 diabetes.

Pathogenesis

Diabetic glomerulosclerosis represents a part of the generalized diabetic microangiopathy that involves small vessels throughout the body (refer page 589-90).

In diabetes, **insulin deficiency, hyperglycemia, (or glucose intolerance) and metabolic defects** cause **biochemical alterations**.

- **Nonenzymatic glycosylation and formation of advanced glycation end-products:** In diabetics, glucose binds to protein nonenzymatically to form advanced glycation end-products (AGEs). These AGEs produce their effects either by binding to its specific receptors (RAGE) or by direct action and includes: **increased synthesis of extracellular matrix, increased production of reactive oxygen species and direct cross-linking with extra-cellular matrix proteins.**
 - **Consequences:** Cross-linking with collagen type IV in glomerular basement membrane (GBM) results in increased collagen type IV and fibronectin and decreased heparin sulfate proteoglycans. This results in **thickening of GBM and increased mesangial matrix.**
- **Hemodynamic changes:** They cause **glomerular hypertrophy** and may also be responsible for the initiation and progression of diabetic glomerulosclerosis. Both **advanced glycosylated end-products and hemodynamic changes** contribute to the **loss of podocytes**. This may occur due to apoptosis of podocytes in response to the metabolic abnormalities and exposure to reactive oxygen species.
- **Disturbances in polyol pathways:** This increases the glomerular cells **susceptibility** to oxidative stress.

Diabetic Nephropathy—Renal Changes in Diabetes

Diabetes: Glomerular lesions may present with:

1. Non-nephrotic proteinuria,
 2. Nephrotic syndrome, and
 3. Chronic renal failure.
- The **kidneys** are **main targets** of diabetes.
 - **Renal failure** is second only to myocardial infarction as a **cause of death** in diabetes.

Renal lesions in diabetes can involve any component. Four lesions are encountered namely:

1. **Glomerular lesions,**
2. **Renal vascular lesions (mainly arteriolosclerosis),**
3. **Pyelonephritis (including necrotizing papillitis)**
4. **Tubular lesions.**

1. Glomerular Lesions (Figs 21.22 and 21.23)

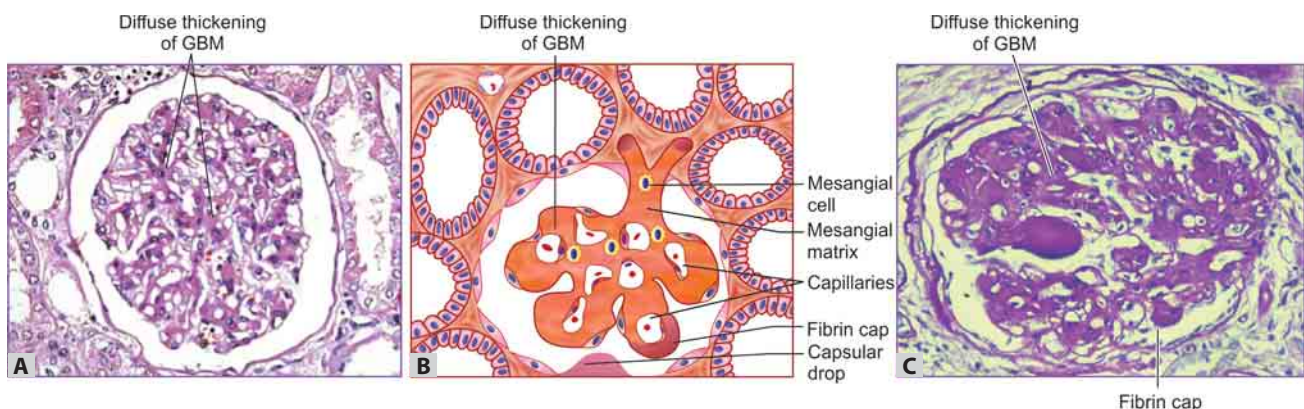
They are most **important and common renal lesions**. These include: (a) Thickening of capillary basement membrane (GBM), (b) diffuse mesangial sclerosis and (c) nodular glomerulosclerosis (Kimmelstiel-Wilson nodules).

- a. **Diffuse widespread thickening glomerular capillary basement membrane:** It occurs in almost all cases of diabetic nephropathy. It represent part and parcel of the diabetic microangiopathy.
- b. **Diffuse mesangial sclerosis:** It is characterized by **diffuse increase in PAS-positive mesangial matrix and mesangial cells**. As the disease progresses, the mesangial areas can expand to assume nodular appearance.
- c. **Nodular glomerulosclerosis:** It is also called as **intercapillary glomerulosclerosis or Kimmelstiel-Wilson disease** and are **pathognomonic**.

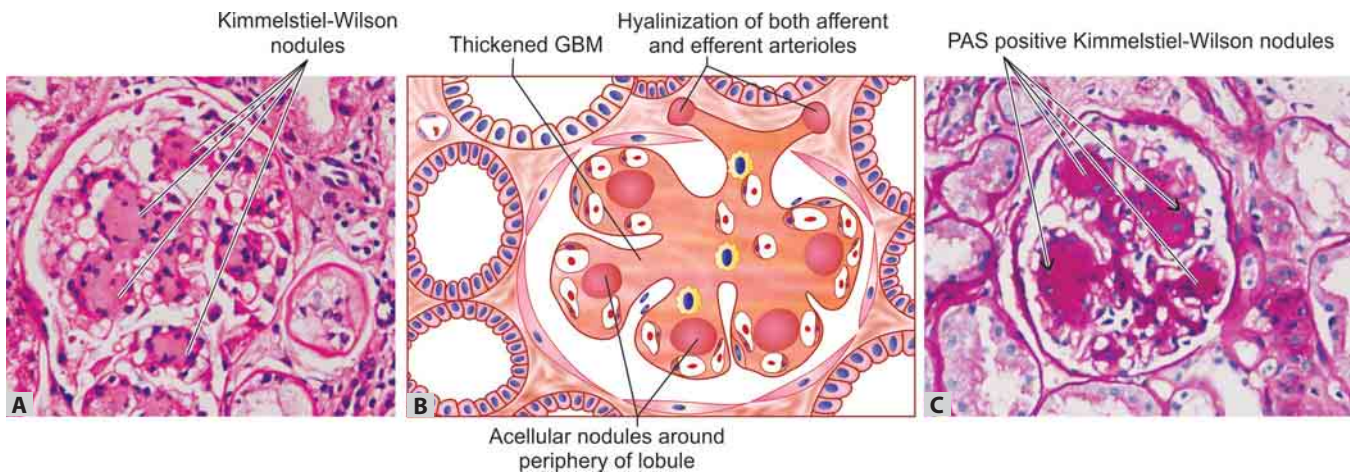
Q. Write short note on Kimmelstiel-Wilson (KW) lesion.

Diabetic nephropathy: Nodular glomerulosclerosis (Kimmelstiel-Wilson nodules) is pathognomonic lesion.

- i. **Develops in about 15–30%** of long-term diabetes and is **mostly associated with renal failure**.
- ii. Glomerular lesion consists of **ovoid or spherical**, often laminated, **nodules of mesangial matrix** seen within the mesangial core and **situated in the periphery of the glomerulus**. These nodules can be surrounded by peripheral capillary loops with patent or dilated lumen. The nodules are **PAS-positive (Fig. 21.23C)**. As the disease advances, the individual nodules increase in size → compress the surrounding capillaries.



Figs 21.22A to C: (A) Hematoxylin and eosin (H & E stain); (B) diagrammatic; and (C) PAS stain, Diabetic nephropathy glomeruli with diffuse widespread thickening glomerular capillary basement membrane and insudative lesions (fibrin cap and capsular drop)



Figs 21.23A to C: Diabetic nephropathy (A) Hematoxylin and eosin (H & E stain); (B) diagrammatic; and (C) PAS stain, nodular glomerulosclerosis shows oval nodules of mesangial matrix at the periphery of glomerulus, C. nodules are PAS positive

d. **Insudative lesions:**

- Fibrin caps:** These are **prominent subendothelial accumulations of hyaline material** in/along capillary loops.
- Capsular drops:** They consist of **round nodules adherent to Bowman's capsules** and are **seen between Bowman's capsule and the parietal epithelium**.

2. Renal Vascular Lesions

- Hyaline arteriosclerosis (hyalinosis):** It involves **both the afferent and the efferent arteriole** at the hilum of glomeruli. **Arteriolosclerosis efferent arteriole is rare in nondiabetics**.
- Renal atherosclerosis:** It involves arteries and arterioles and are similar to those found in other parts of the body. Glomerular and arteriolar lesions together produce **renal ischemia** → leads to atrophy of tubules, interstitial fibrosis, and contraction of kidney.

3. Pyelonephritis

It is a tubulointerstitial inflammation of the kidneys.

- Both the **acute and chronic pyelonephritis** occurs in nondiabetics as well as in diabetics. It is **more common** and tends to **more severe** in diabetics than in the general population.
- Necrotizing papillitis** (or papillary necrosis): It is one **special pattern of acute pyelonephritis** and is much more frequent in diabetics compared to nondiabetics. **Hyaline arteriolosclerosis** narrows blood vessels → **reduces the blood supply** to the renal medulla → ischemia → causes **necrosis of the tips of papillae** (papillary necrosis).

Causes of necrotizing papillitis:

- Diabetes mellitus
- Sickle cell anemia
- Analgesic nephropathy
- Obstructive uropathy.

4. Tubular Lesions

Armani-Ebstein lesion: Proximal tubular epithelial cells with glycogen accumulation seen in uncontrolled diabetes mellitus.

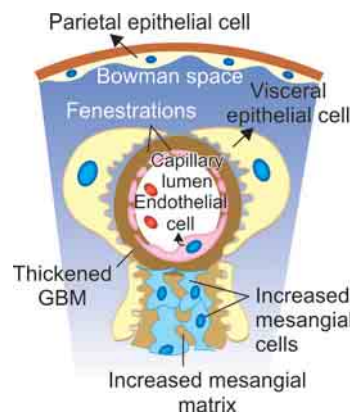
The basement membrane of the tubules show thickening. In patients with high blood sugar level, the **epithelial cells of the proximal convoluted tubules** show extensive deposits of **vacuoles of glycogen**. These are known as **Armani-Ebstein lesions**.

Electron Microscopy (Fig. 21.24)

- **Thickening of the lamina densa** of the glomerular basement membrane.
- **Increase in the mesangial matrix**, particularly in nodular lesions.
- **Insudative lesions** appear as electron-dense masses.

Immunofluorescence Microscopy

It reveals IgG, albumin, fibrinogen, and other **plasma proteins** in the GBM.



Microalbuminuria: Urinary excretion of 30 to 300 mg of albumin/24 hours.

Diabetic glomerulopathy: Microalbuminuria is first laboratory sign.

Fig. 21.24: Diagrammatic electron microscopic appearance of diabetic glomerulosclerosis showing thickened glomerular basement membrane, increased mesangial cells and mesangial matrix

Amyloidosis

Kidney is the most common and the most serious form of organ involvement in amyloidosis (Renal amyloidosis—refer page 158).

PYELONEPHRITIS AND URINARY TRACT INFECTION

Classification of Urinary Tract Infections

- **Lower urinary tract infections:** These include cystitis (bladder) and urethritis. Bacterial infection of the lower urinary tract may be completely asymptomatic (asymptomatic bacteriuria). Most of the cases, it remains localized to the bladder. However, lower urinary tract infection always carries the risk of spread to the kidney.
- **Pyelonephritis:** It involves kidneys and their collecting systems (pyelonephritis).

PYELONEPHRITIS

Definition: Pyelonephritis is inflammatory disease of kidney affecting the tubules, interstitium, and renal pelvis. It is one of the most common diseases of the kidney.

Classification of Pyelonephritis

- **Acute pyelonephritis:** It is caused by bacterial infection and is associated with urinary tract infection.
- **Chronic pyelonephritis:** It is a more complex disorder. However, bacterial infection plays a major role, but other factors (vesicoureteral reflux, obstruction) are also involved in its pathogenesis.

Acute Pyelonephritis

Acute pyelonephritis is an acute suppurative inflammation of the kidney affecting the tubules, interstitium, and renal pelvis.

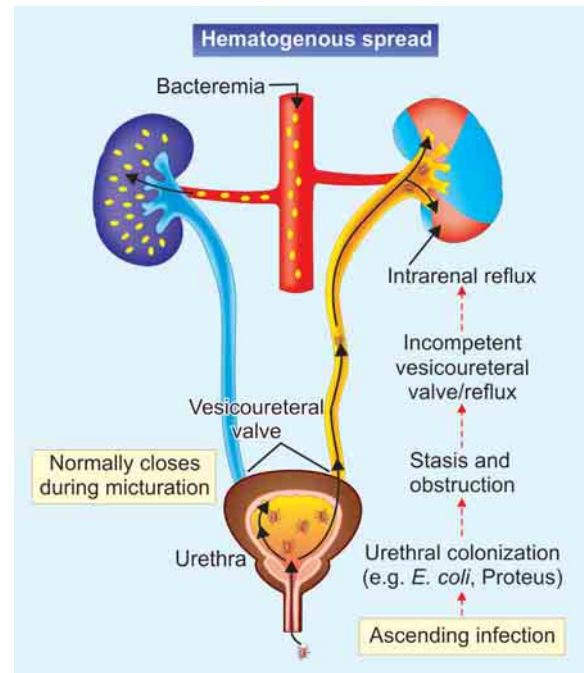
Etiology and Pathogenesis (Fig. 21.25)

Q. Write short note on etiopathogenesis of acute pyelonephritis.

Causative Organisms

Acute pyelonephritis: Bacterial infection of renal tubules, interstitium and renal pelvis.

Majority (~85%) of urinary tract infection are caused by **Gram-negative bacilli**, which are normal inhabitants of the intestinal tract (enteric origin).



Pyelonephritis: Ascending infection is the most common route of infection by most bacteria.

Acute pyelonephritis: Caused by ascending bacterial infection or hematogenous spread.

Fig. 21.25: Pathogenesis of acute pyelonephritis. More common mode is ascending infection. Hematogenous infection results from bacteremic spread

- **Most common pathogens:** *Escherichia coli*, *Proteus*, *Klebsiella*, and *Enterobacter*.
- **Less common:** *Streptococcus faecalis*, staphylococci, and fungi.
- **In immunocompromized patients:** Viruses (polyomavirus, cytomegalovirus, and adenovirus).

Acute pyelonephritis: Predisposing conditions

1. Urinary tract obstruction
2. Instrumentation of the urinary tract
3. Pregnancy
4. Female gender
5. Diabetes mellitus
6. Immunosuppression and immunodeficiency
7. Urinary catheters.

Source and Route of Infection

Normal urine in the bladder is sterile.

Bacteria can reach the kidney by **two routes**:

- A. Ascending infection:** It is the **most common route** of infection of pyelonephritis. It is a form of endogenous

infection, where the source of infecting organisms is the **patient's own fecal flora**. The infection ascends from the lower urinary tract into the renal parenchyma. **Different steps** in the pathogenesis of pyelonephritis are:

1. **Colonization of the distal urethra and introitus** (in the female): By enteric or coliform bacteria from the perineum due to poor hygiene and hormonal effects.
2. **Entry from the urethra to the bladder**: Organisms may enter to the bladder during **urethral catheterization or other instrumentation**. Urinary infections are **more common in females**, because of:
 - Shorter urethra
 - Absence of prostatic fluid, which has antibacterial properties.
 - Hormonal changes in women, which affect the adherence of bacteria to the mucosa.
 - Trauma to the urethra during sexual intercourse.

If organisms enter into the bladder, they are normally cleared by the continual flushing of voiding and by antibacterial mechanisms.

3. **Urinary tract obstruction and stasis of urine**:
 - **Obstruction/bladder dysfunction**: It causes **incomplete emptying and increased residual volume of urine**. Examples:
 - **Lower urinary tract obstruction**: For example, Benign prostatic hypertrophy, tumors, or calculi.
 - **Neurogenic bladder dysfunction**: For example, diabetes or spinal cord injury.
 - **Stasis**: **Organisms** introduced into the bladder can **multiply** when there is stasis.

Normal ureteral insertion into the bladder is in such a way that it prevents retrograde flow of urine during micturition or whenever there is raised pressure in the bladder.

4. **Vesicoureteral reflux**: It **propels infected bladder urine into the renal pelvis** through ureter. Incompetent vesicoureteral orifice/valve may be congenital or acquired.
 - **Congenital vesicoureteral reflux**: For example, congenital absence or shortening of the intravesical portion of the ureter
 - **Acquired vesicoureteral reflux**: For example, persistent bladder atony caused by spinal cord injury.

Renal papillae in the midzones are convex pointed type and do not allow urine to flow back into the collecting tubules.

5. **Intrarenal reflux**: It is most common at both the poles of the kidney and allow the organism to enter from the renal pelvis into the renal parenchyma through open ducts at the tips of the papillae.

Renal papillae in the upper and lower poles tend to have flattened or concave tips and can allow urine to flow back into the collecting tubules.

- B. **Hematogenous route**: It is **less common route of infection**. Because of rich blood supply, bacteria can seed the kidneys during the **course of septicemia or infective endocarditis** through the bloodstream. It occurs with **nonenteric organisms** (e.g. staphylococci), fungi and viruses. Hematogenous infections occur in:
 - The presence of ureteral obstruction
 - Debilitated patients
 - Patients receiving immunosuppressive therapy.

Tuberculosis: Involves kidney by hematogenous spread.

MORPHOLOGY

Gross

- **Unilateral or bilateral**.
- **Focal abscesses**: It may be seen on the **subcapsular surface** that appear small and **white**. In pyelonephritis associated with reflux, they are **most common in the lower and upper poles**.
- **Pelvic and calyces**: They may be **hyperemic and covered by purulent exudate**.

Microscopy (Fig. 21.26)

- **Interstitial**: It shows **patchy interstitial neutrophilic infiltration**, which may later become extensive.
- **Tubules**: **Intratubular aggregates of neutrophils** forms abscess with the destruction of the involved tubules → **tubular necrosis**.
- **Glomeruli**: They appear normal because they are relatively resistant to the infection.

Complications

Q. Write short note on complications of acute pyelonephritis.

Complications of acute pyelonephritis:

1. Papillary necrosis
2. Pyonephrosis
3. Perinephric abscess.

1. **Papillary necrosis**: It is the **necrosis of the tips of the renal papillae**.
 - Seen mainly in **diabetics** and with **urinary tract obstruction**.
 - **Usually bilateral** but may be unilateral. One or all of the pyramids of the affected kidney may be involved.
 - **Cut section**: The **tips or distal two-thirds of the renal pyramids** show areas of gray-white to yellow **necrosis**.
 - **Microscopy**: The necrotic area shows **coagulative necrosis**, with preservation of tubular outline.

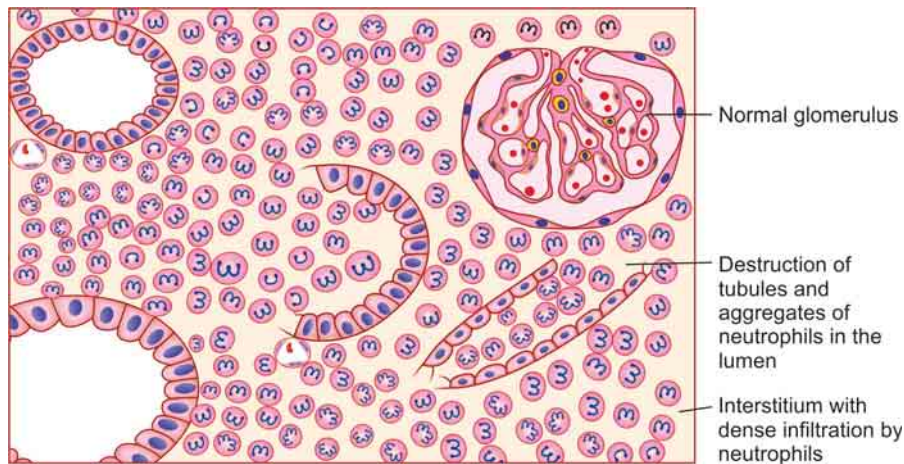


Fig. 21.26: Acute pyelonephritis with neutrophilic exudate within tubules and interstitium

2. **Pyonephrosis:** It is characterized by accumulation of **pus** (suppurative exudates) **within the renal pelvis, calyces, and ureter** → kidney distended with pus. This complication develops when there is total or complete obstruction to the urinary drainage high in the urinary tract near the kidney.
3. **Perinephric abscess:** It develops when the **suppurative infection breaks the renal capsule** and spread into the perinephric tissue.

Clinical Features

- Sudden onset of pain at the costovertebral angle, fever and malaise.
- Dysuria, frequency and urgency.

Urinary Findings

Microscopy

- **Pus cells:** Urine shows many leukocytes (**pyuria**); but it does not differentiate upper from lower urinary tract infection.
- **WBC casts:** Casts are formed only in tubules and the finding of leukocyte casts, which is rich in neutrophils (pus cells), **indicates renal involvement** (i.e. pyelonephritis).

WBC casts in urine indicates pyelonephritis because casts are formed only in tubules.

Culture and sensitivity: It can establish the causative organism.

Sterile pyuria: White blood cells in urine in the absence of significant bacterial growth.

Course of the disease: Usually, follows a benign course.

Chronic Pyelonephritis and Reflux Nephropathy

Q. Describe the etiopathogenesis of chronic pyelonephritis.

Definition: Chronic pyelonephritis is a **chronic inflammation of tubulointerstitial tissue** leading to scarring of calyces, pelvis and renal parenchyma.

Chronic pyelonephritis is an **important cause of end-stage renal disease**.

Types of chronic pyelonephritis: Chronic reflux-associated pyelonephritis or chronic obstructive pyelonephritis.

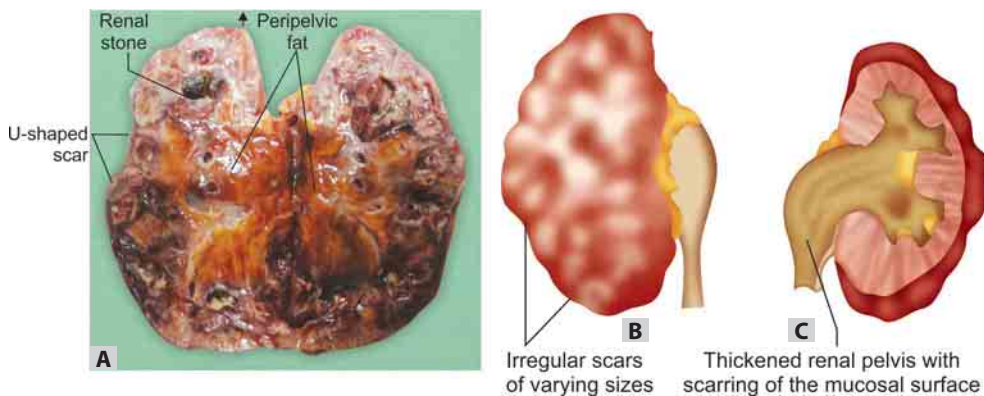
1. **Reflux nephropathy (chronic reflux-associated pyelonephritis)**
 - Develops due to **superimposition of a urinary infection on congenital vesicoureteral reflux and intrarenal reflux**.
 - Reflux may be unilateral or bilateral and accordingly causes scarring of one or both kidney, leading to chronic renal insufficiency.
2. **Chronic obstructive pyelonephritis**
 - Develops due to **recurrent infections superimposed on obstructive lesions**, which lead to renal inflammation parenchymal atrophy and scarring.

MORPHOLOGY

Q. Describe gross and microscopy of chronic pyelonephritis.

Gross (Fig. 21.27)

- May be **unilateral or bilateral**. When bilateral, the involvement is **asymmetric**.
- **Size:** Kidney is **reduced** in size due to **irregular scars**.



Chronic pyelonephritis: If bilateral, involvement is asymmetric. In chronic glomerulonephritis, both kidneys are diffusely and symmetrically scarred.

Chronic pyelonephritis: Scarring of kidney → granular contracted kidney.

Figs 21.27A to C: Gross appearance of chronic pyelonephritis. (A) Cut section of kidney showing scars and a renal stone in the calyces; (B) (diagrammatic) Outer aspect; (C) Cut section (diagrammatic)

- **Nature of scars:** May affect one or both kidneys.
 - Most are seen in the **upper and lower poles** due to intrarenal reflux.
 - Scars are **coarse, well-defined** and can vary from one to several in number.
- **Calyces and renal pelvis:** It may appear **thickened and irregular with scarring of the mucosal surface**. Calyces below the scars are **dilated, blunted, or deformed**. Papillae are **flattened**.

Microscopy (Fig. 21.28)

Changes are predominant in the interstitium and tubules.

- **Interstitium:** It shows dense **chronic inflammatory infiltrate** of lymphocytes and macrophages and fibrosis. Few neutrophils may be found with active infection.
- **Tubules:** It may show **atrophy** in some places and hypertrophy or dilation in others.
 - **Thyroidization:** Dilated tubules with flattened epithelium may be **filled with eosinophilic hyaline material and resemble colloid** containing thyroid follicles.
 - **Blood vessels:** They may show **endarteritis obliterans** and in the presence of hypertension may show **hyaline arteriosclerosis**.
- **Glomeruli:** It may appear normal except for **periglomerular fibrosis**.
- **Calyces:** Shows calyceal epithelium surrounded by **fibrosis** and infiltrated by **dense chronic inflammatory infiltrate**.

Periglomerular fibrosis: Characteristic of chronic pyelonephritis.

Xanthogranulomatous Pyelonephritis

It is an **unusual and rare form of chronic pyelonephritis** associated with *Proteus* infections.

MORPHOLOGY

- **Gross:** Cut surface shows large, yellowish-orange nodules, which may be **confused with renal cell carcinoma**.
- **Microscopy:** Interstitium shows foamy **macrophages, plasma cells and lymphocytes** (Fig. 21.28C).

Clinical Features

Chronic pyelonephritis associated with reflux **may be silent** onset. Usual symptoms are **back-pain, fever, pyuria and bacteriuria**.

BENIGN NEPHROSCLEROSIS

Q. Write short note on benign nephrosclerosis.

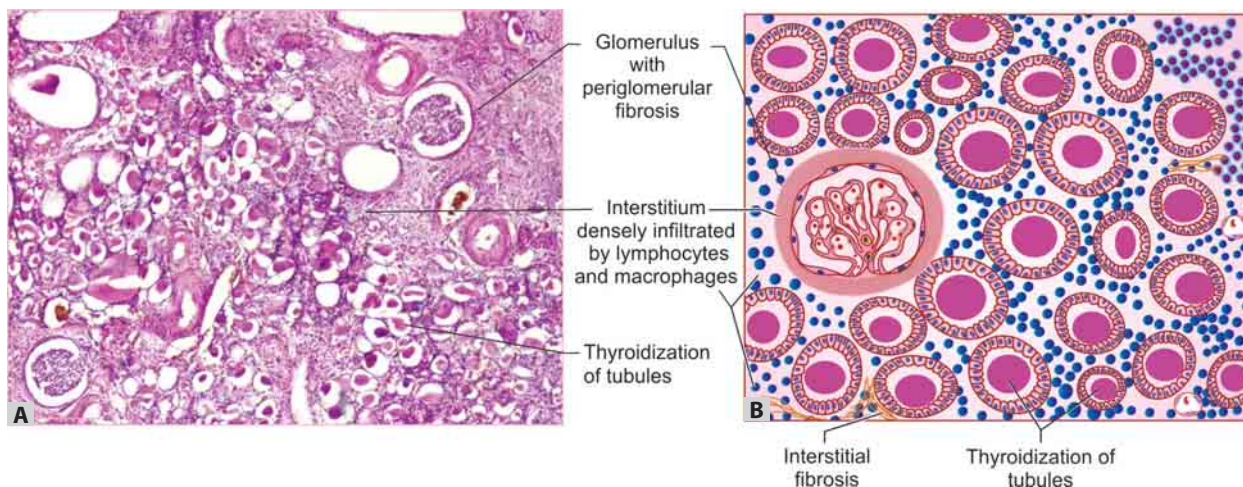
Definition: Benign nephrosclerosis is defined as the **renal pathology associated with sclerosis of renal arterioles and small arteries**.

- Sclerosed vessels with thickened walls → cause narrowing of lumens → result in focal ischemia of renal parenchyma → glomerulosclerosis + chronic tubulointerstitial injury → reduction in functional renal mass.

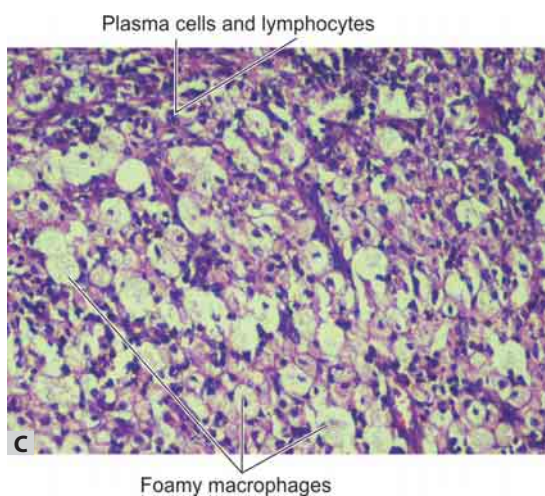
Causes

Benign nephrosclerosis is associated with:

- Hypertension
 - Diabetes mellitus
 - Increasing age and may be seen in the absence of hypertension.
- } increases the incidence and severity of the lesions



Q. Write short note on granular contracted kidney.



Chronic pyelonephritis:

- Thyroidization
- Chronic inflammatory infiltrate
- Periglomerular fibrosis.

Granular contracted kidney due to chronic pyelonephritis: Unilateral or bilateral; when bilateral asymmetrically involved. The scars are large, irregular and coarse.

Causes of granular contracted kidney:

- Chronic glomerulonephritis
- Chronic pyelonephritis
- Benign nephrosclerosis.

Granular contracted kidney due to chronic glomerulonephritis and benign nephrosclerosis: Bilateral symmetrically involvement with fine, uniform and small, granular scars. Scars are V-shaped in benign nephrosclerosis whereas U-shaped in chronic glomerulonephritis.

Figs 21.28A to C: Microscopic appearance of chronic pyelonephritis. Tubules containing hyaline material resembling the colloid of thyroid follicles (thyroidization), interstitium with chronic inflammatory cell infiltrate and periglomerular fibrosis; (A) Inset (left lower) shows glomerulus with periglomerular fibrosis; (B) Diagrammatic; (C) Xanthogranulomatous pyelonephritis showing foamy macrophages, plasma cells and lymphocytes

Pathogenesis

Two processes which results in the arterial lesions are:

- **Medial and intimal thickening:** It develops as a response to hemodynamic changes, aging, genetic defects, or any combination of these factors.
- **Hyaline deposition in arterioles:** It is partly due to extravasation of plasma proteins through injured endothelium and partly due to increased deposition of basement membrane matrix.

Hypertension is closely linked with the kidney. Kidney can be both a target and a cause of hypertension.

MORPHOLOGY

Q. Write short note on kidney changes in hypertension.

Gross

- **Involvement:** Bilateral.
- **Size:** Kidneys are either normal or smaller (atrophic).
- **Weight:** Between 110 and 130 g (normal 150 g).
- **Outer surface:** It shows a fine, even granularity resembling grain leather.
- **Cut section:** It shows thinned cortex with scarring.

Microscopy

Benign nephrosclerosis:

- Hyaline arteriolosclerosis
- Fibroelastic hyperplasia of blood vessels.

1. **Blood vessel:** Changes in the kidney depend on the size of the vessel.
 - **Arterioles and small arteries:** They show thickening and hyalinization of the walls causing narrowing of their lumens called as **hyaline arteriolosclerosis** (refer Fig. 14.8).
 - **Interlobular and arcuate arteries:** They show a characteristic **fibrotic thickening of intima with reduplication of the elastic lamina, medial hypertrophy** known as **fibroelastic hyperplasia** → narrow the lumen.
- Consequences of vascular narrowing** → foci of tubular atrophy and interstitial fibrosis + glomerular alterations.
2. **Tubules:** They show atrophy.
 3. **Interstitium:** Fibrosis and chronic inflammatory infiltrate.
 4. **Glomeruli:** Most of them appear normal and others may show.
 - **Thickening of glomerular capillaries:** It is due to **thickening, wrinkling and collapse of GBMs**.
 - **Sclerosis of glomeruli.**

Primary disease of the kidney is the most common cause of secondary hypertension.

Clinical Features

Uncomplicated benign nephrosclerosis does not cause renal insufficiency or uremia. There are usually associated with moderate reductions in renal blood flow, but the GFR is either normal or slightly reduced.

MALIGNANT HYPERTENSION AND ACCELERATED NEPHROSCLEROSIS

Definition: Malignant nephrosclerosis is defined as **renal disease associated with the malignant or accelerated phase of hypertension**.

- Malignant or accelerated phase of hypertension is relatively uncommon.
- It is often superimposed on pre-existing **essential benign hypertension, secondary forms of hypertension**, or an underlying chronic renal disease, particularly glomerulonephritis or reflux nephropathy.
- Occasionally, it may develop in previously **normotensive** individuals.
- **Age and sex:** Pure form usually seen in younger age and more often in men.

Pathogenesis

Exact pathogenesis is not known, but the following sequence of events is suggested.

- **Vascular damage to the kidneys:** It may be by the initial insult (e.g. long-standing benign hypertension) and may result in:
 - **Increased permeability of the small vessels** (arterioles and small arteries) to fibrinogen and other plasma proteins → leads to **fibrinoid necrosis**.
 - **Endothelial injury and deposition of platelets** leads to **swelling of the vascular intima and intravascular thrombosis**.
- **Hyperplastic arteriolosclerosis:** Mitogenic factors from platelets (e.g. PDGF), plasma and other cells cause **hyperplasia (proliferation) of intimal smooth muscle** → **hyperplastic arteriolosclerosis** characteristics of **malignant hypertension** → **narrow the vascular lumen** → results in **ischemia of the kidneys**.
- **Raised plasma rennin:** Severe narrowing of renal afferent arterioles → stimulation of **renin-angiotensin system** → **raised levels of plasma renin**.
- **Raised blood pressure:** Elevated aldosterone levels → salt retention → elevation of blood pressure → **changes in blood vessels throughout the body** known as **malignant arteriosclerosis**.

Malignant nephrosclerosis: Renal changes due to malignant hypertension.

Renin: Major source is juxtaglomerular (JG) cell of the kidney.

MORPHOLOGY**Gross**

- The size of the kidney depends on the duration and severity of the hypertension.
- **Flea-bitten kidney:** It is characterized by the presence of **small, pinpoint petechial hemorrhages on the cortical surface** due to **rupture of arterioles or glomerular capillaries**.

Microscopy

Malignant nephrosclerosis: Fibrinoid necrosis and onion-skinning of arterioles.

Histological changes of blood vessels → narrowing of vascular lumens → ischemic atrophy.

- **Fibrinoid necrosis of arterioles:** It is characterized by **eosinophilic granular change in the wall of the blood vessel**. It stains positively for fibrin by histochemical or immunofluorescence techniques.
- **Onion-skinning** (hyperplastic arteriolitis) (refer Fig. 14.8B): It is seen in the **interlobular arteries and arterioles**. It is characterized by **intimal thickening due to concentric proliferation of smooth muscle cells + fine concentric layering of collagen, proteoglycans and plasma proteins**.

Causes of flea bitten kidney:

1. Malignant hypertension
2. Subacute bacterial endocarditis
3. Rapidly progressive GN
4. Henoch-Schönlein purpura.



Fig. 21.29: Horseshoe kidney showing fusion of the lower poles

Clinical Features

- Malignant hypertension is characterized by systolic pressures more than 200 mm Hg and diastolic pressures more than 120 mm Hg, papilledema, retinal hemorrhages, encephalopathy, cardiovascular abnormalities and renal failure.
- Initial symptoms are due to raised intracranial pressure and include headaches, nausea, vomiting and visual impairments.
- Urine examination may show marked proteinuria and hematuria without any significant alteration in renal function.
- Later renal failure may develop.

HORSESHOE KIDNEYS

Q. Write short note on horseshoe kidney.

- Horseshoe kidneys are **common congenital anomaly** in which there is fusion of the upper (10%) or lower poles (90%) of the kidneys.
- It gives a horseshoe appearance to kidneys (Fig. 21.29), which are continuous across the midline anterior to the great vessels.

CYSTIC DISEASES OF THE KIDNEY

Definition: Cystic diseases of the kidney represent a heterogeneous group of hereditary, developmental, and acquired disorders characterized by the presence of unilateral or bilateral renal cysts.

Classification of Renal Cystic Disease (Box 21.6)

Autosomal Dominant (Adult) Polycystic Kidney Disease

Q. Write short note on polycystic kidney disease.

Definition: Autosomal-dominant (adult) polycystic kidney disease (ADPKD) is a **hereditary disorder** characterized

BOX 21.6: Classification of renal cystic disease

1. Polycystic kidney disease
 - Autosomal dominant (adult) polycystic disease
 - Autosomal recessive (childhood) polycystic disease
2. Medullary cystic disease
 - Medullary sponge kidney
 - Nephronophthisis
3. Multicystic renal dysplasia
4. Acquired (dialysis-associated) cystic disease
5. Localized (simple) renal cysts
6. Renal cysts in hereditary malformation syndromes (e.g. tuberous sclerosis)
7. Glomerulocystic disease
8. Extraparenchymal renal cysts (pyelocalyceal cysts, hilar lymphangitic cysts)

by **multiple cysts in both kidneys**. These cysts expand and cause destruction of kidney parenchyma and leads to renal failure.

ADPKD: Autosomal dominant mode of inheritance.

Genetics and Pathogenesis

Genetics

ADPKD: Mutations in

1. *PKD1* gene on chromosome 16p13.3 code for polycystin-1, or
2. *PKD2* gene on chromosome 4q21 code for polycystin-2.

- The ADPKD is **genetically heterogeneous disorder** caused by **mutations in *PKD1*** (85% of cases) and ***PKD2* gene** (15%).
- Germline mutations in *PKD1* gene are present in all renal tubular epithelial cells of affected individuals, cyst only develop in some tubules.
- Like all tumor suppressor genes, *PKD* gene also requires a second hit for the expression of disease.
- Mutations in other genes involved in polycystic kidneys include gene, which produce protein fibrocystin/polyductin and nephrocystin.

Pathogenesis (Fig. 21.30)

The exact pathogenesis of ADPKD is unknown.

- Protein products of *PKD* genes are **polycystin-1** and **polycystin-2**. The polycystins are mainly extracellular molecules present in the primary cilia of tubular epithelial cells that are **involved in cell-cell or cell-matrix adhesion**. Cilia are hair-like organelles present on the apical surface of tubular epithelial cells. They **act as mechanosensors of fluid flow in the lumen of the tubules**.
- Renal cystic disease is considered as a type of **ciliopathy**.

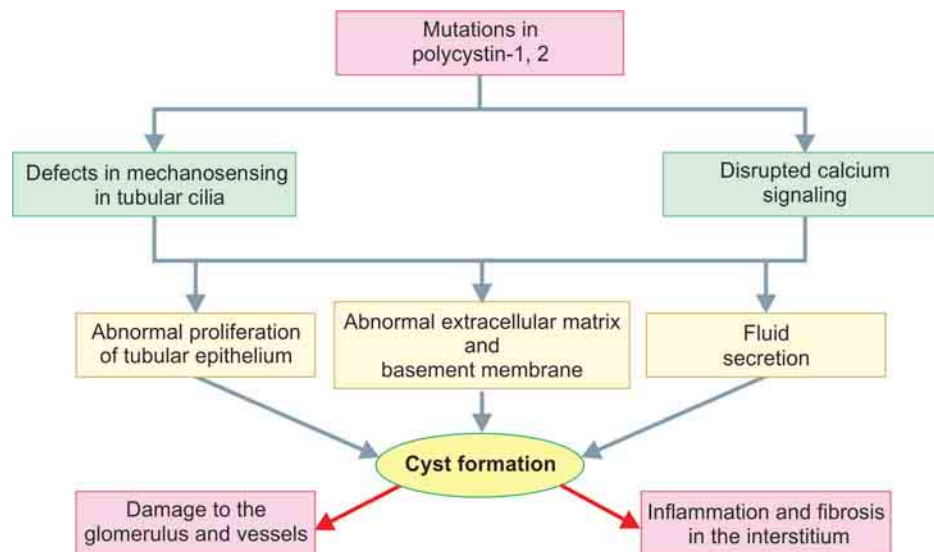


Fig. 21.30: Mechanisms of cyst formation in polycystic kidney disease

- **Mutations of the *PKD* genes lead to defects in mechanosensing.** This causes **disrupted calcium signaling** (cilium is essential for maintenance of epithelial cell differentiation and normally inhibits renal tubule growth) and leads to **dysregulation of cell polarity, abnormal cell proliferation and cell-matrix adhesion.**
- **Evolution of cysts from renal tubules**
 - **Abnormal proliferation of tubular epithelium:** It follows disrupted calcium signaling and tubule becomes covered by an undifferentiated epithelium. **Repeated cycles of cell proliferation lead to dilatation/expansion of the tubule wall into a cyst.**
 - **Abnormal extracellular matrix and defect in basement membrane:** The cystic epithelium is associated with **thickening of the basement membrane of the affected tubule.**
 - **Fluid secretion:** Initially, the cyst fluid is derived from the glomerular filtrate. Later, the cystic segment separates (becomes disconnected) from the original tubule, and the fluid is secreted by epithelial cells lining the cysts into the cyst cavity.
- **Progression of cyst size:** The abnormal proliferation of tubular epithelial cells and the expanding volume of intraluminal fluid result in progressive increase in the size of the cysts.
 - **Consequences:** The cyst fluids contain mediators derived from epithelial cells, which further promotes fluid secretion, damage glomerulus, stimulate inflammation and interstitial fibrosis.

MORPHOLOGY

Gross (Fig. 21.31)

- **Bilateral.**
- **Size: Markedly enlarged;** weight is increased and each may reach up to 4 kg.
- **External surface: Bosselated with numerous cysts of varying sizes,** measuring up to 3 to 4 cm in diameter, with no intervening parenchyma.

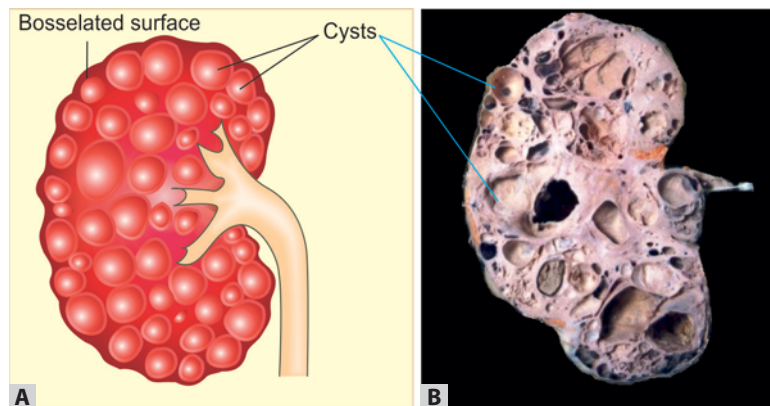
Microscopy

- **Numerous cysts** with functioning nephrons between these cysts.
- **Cysts:**
 - **Origin:** Cysts can arise in all segments of the nephron including glomeruli, proximal tubules, distal tubules, and collecting ducts, and therefore, they **show variable lining epithelia.** Occasionally, Bowman capsules may be involved in cyst formation.
 - **Lining:** **Cuboidal and columnar** epithelium.
 - **Content:** Cysts may show a **clear, serous fluid or more commonly contain turbid, red to brown,** sometimes hemorrhagic fluid.
- **Intervening renal parenchyma:** It may be normal or may show foci of interstitial fibrosis and pyelonephritis.

Clinical Features

ADPKD: Chronic renal failure develops between ages of 40 to 60 years.

- **Majority of patients remain asymptomatic until the fourth decade of life.**



Figs 21.31A and B: Autosomal-dominant adult polycystic kidney disease (ADPKD) shows markedly enlarged kidney with numerous dilated cysts. (A) External surface (Diagrammatic); (B) Cut section

- It may present with **flank pain** (heaviness or dragging sensation), **bilateral abdominal masses**, and **renal colic** (due to passage of blood clots in the urine), and **hypertension**. When sufficient quantity of nephrons are destroyed, patient develops **renal failure**.

Extrarenal Associated Congenital Anomalies

ADPKD:

1. Large multicystic kidneys, liver cysts
 2. Berry aneurysms
 3. Mitral valve prolapse.
- Cysts in other organs such as liver (**polycystic liver disease**).
 - Intracranial **berry aneurysms** in the **circle of Willis** which may rupture and cause subarachnoid hemorrhages.
 - **Mitral valve prolapse** and other cardiac valvular anomalies.

Autosomal Recessive (Childhood) Polycystic Kidney Disease

Autosomal recessive (childhood) polycystic kidney disease (ARPKD) is **genetically different** from adult polycystic kidney disease (ADPKD).

Childhood polycystic kidney disease: Autosomal recessive.

Subcategories: ARPKD can be categorized as **perinatal**, **neonatal**, **infantile**, and **juvenile**, depending on the time of presentation. The perinatal and neonatal categories are most common and are usually fatal.

Etiology

ARPKD: Mutations in gene *PKHD1*, which codes for fibrocystin.

- In **majority** of the cases, ARPKD is caused by **mutations of the polycystic kidney hepatic disease gene (*PKHD1*) gene**.
- *PKHD1* **encodes for a protein called fibrocystin (polyductin)**.
- It is highly expressed in the epithelial cells of the collecting ducts and thick ascending loops of Henle.

MORPHOLOGY

Gross

ARPKD: Large, cystic kidneys at birth.

- **Both kidneys are markedly enlarged** and have a **smooth external appearance**. Thus, they retain their **reniform configuration**.
- **Cut section** (Fig. 21.32):
 - **Medulla and cortex are completely replaced by numerous small cysts**.
 - The **cysts tend to be linear and radiate from the medulla to the outer cortex** (right angles to the cortical surface). It gives a **sponge-like appearance** to the kidney.

Microscopy

- **Shape:** Cyst is **cylindrical** and appears as dilated tubular structures or, less commonly saccular.
- **Lining:** **All the cysts are lined by cuboidal epithelial cells**, reflecting their **origin from the collecting ducts**.

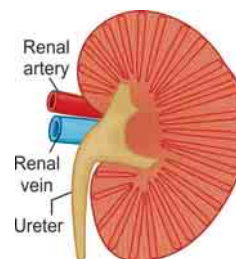


Fig. 21.32: Diagrammatic appearance of cut section of autosomal-recessive childhood PKD, showing smaller cysts and dilated channels at right angles to the cortical surface

Features of ARPKD

ARPKD: Usually death in infancy or childhood.

- All patients also have cysts in the liver.
- Patients who survive infancy (infantile and juvenile forms), may develop **congenital hepatic fibrosis**.
- It is characterized by bland periportal fibrosis and the proliferation of well-differentiated biliary ductules.
- Older children may develop portal hypertension with splenomegaly.

ACUTE KIDNEY INJURY

AKI/ATN: Most common cause of acute renal failure.

Acute kidney injury (AKI), is also known as **acute tubular necrosis (ATN)** and **acute tubular injury (ATI)**.

Definition: AKI is a **clinicopathologic entity characterized clinically by acute reduction of renal function** and mostly with morphological feature of tubular injury.

It is the **most common cause of acute renal failure**. Acute renal failure is characterized by rapid reduction of renal function and with severe oliguria (urine less than 400 mL per day).

Causes of Acute Kidney Injury (Box 21.7)

AKI/ATN: Most common is ischemic type.

AKI: Acute reduction of renal function and mostly with morphological feature of tubular injury.

Pathogenesis (Fig. 21.33)

Pathogenesis of AKI:

1. Tubular cell injury
2. Disturbances in blood flow.

Two important features of AKI (in both ischemic and nephrotoxic) are: (1) Tubule cell injury, and (2) Disturbances in blood flow.

1. Injury to the Tubular Epithelial Cells

Tubular cells are sensitive to both ischemia and toxins.

- a. **Ischemia:** Ischemia may cause **reversible injury** (such as swelling, blebbing) or **irreversible injury** (necrosis and apoptosis). **Tubular necrosis** is **focal** and **multiple**, and the **tubular basement membrane** remains **intact**. If the precipitating cause is removed, repair of the necrotic foci and recovery of function can occur.

BOX 21.7: Causes of acute kidney injury

- **Ischemia:** Due to decreased or interrupted blood flow and is known as *ischemic AKI*.
 - **Reduction of effective circulating blood volume:**
 - ◆ Loss of blood: Massive hemorrhage
 - ◆ Loss of fluid: Severe burns, dehydration, prolonged diarrhea
 - ◆ Septic shock
 - ◆ Congestive heart failure
 - **Diffuse involvement of the intrarenal blood vessels:**
 - ◆ Microscopic polyangiitis
 - ◆ Malignant hypertension
 - ◆ Systemic conditions: For example, disseminated intravascular coagulation (DIC)
- **Direct toxic injury to the tubules (nephrotoxic AKI)**
 - Antibiotics (e.g. aminoglycosides, gentamicin, amphotericin B)
 - Poisons such as heavy metals (e.g. mercury, lead, cisplatin)
 - Organic solvents (e.g. carbon tetrachloride, ethylene glycol)
 - Radiation
- **Combinations of ischemic and nephrotoxic AKI**
 - Mismatched blood transfusions
 - Hemolytic crises: For example, sickle cell anemia
 - Skeletal muscle injuries causing myoglobinuria
- **Acute tubulointerstitial nephritis:** Hypersensitivity reaction to drugs
- **Urinary obstruction** (postrenal acute renal failure)
 - Tumors
 - Prostatic hypertrophy

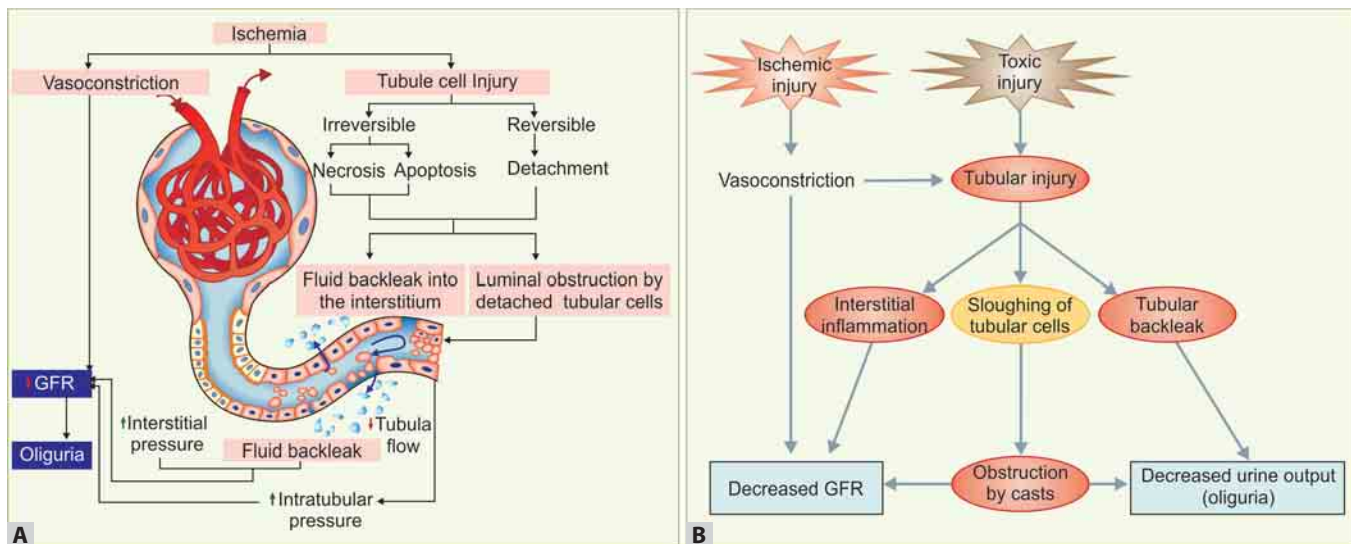
AKI is **NOT** caused by TB.

AKI: Two important types
1. Ischemic 2. Toxic.

- b. **Toxins:** They can cause **direct injury to tubular epithelial cells**.

Consequences of Tubular Injury

- **Back-leakage of fluid from lumen into the interstitium:** It occurs in the **damaged tubules** → result in **interstitial edema** → causes **increased interstitial pressure**, and **further damage to the tubule**.
- **Luminal obstruction by casts:** Tubular epithelial cells gets detached from injured tubules and form casts leading to obstruction of tubular lumen. **Obstruction results in:** (1) **increased intratubular pressure**, (2) **decreased GFR**, and (3) **decreased urine outflow**.
- **Interstitial inflammation:** It also causes decreased GFR.



Figs 21.33A and B: Pathogenesis of acute kidney injury

2. Disturbances in Blood Flow

Ischemia also causes **vasoconstriction** (intrarenal) and reduces both **glomerular blood flow** and **oxygen supply** to tubules.

MORPHOLOGY

Ischemic AKI

Gross

- Both kidneys are **swollen** and show a **pale cortex** and a **congested medulla** (Fig. 21.34).

Microscopy

1. **Glomeruli:** Appear normal.

2. **Tubules:**

- Areas involved** (Fig. 21.35A): Tubules show **focal and multiple areas of damage** along the nephron, with **large skip areas** in between. The lesions are **most marked in the proximal tubules** and the **ascending thick limbs of the loop of Henle in the outer medulla**.
- Tubular epithelial injury:**
 - Epithelial simplification:** It is characterized by **focal flattening** and **loss of proximal tubule brush borders**.
 - Sloughing** of the apical cytoplasm of non-necrotic tubular cells into the lumen of the tubules.
 - Necrosis of tubular epithelial cells** (Fig. 21.36).
 - Formation of casts:** Sloughed apical cytoplasm, non-necrotic and necrotic cells form **hyaline casts** and **brown pigmented granular cast**. The casts are seen in the lumen of distal tubules and in the urine. These casts are **composed of Tamm-Horsfall protein** (a urinary glycoprotein normally secreted by the cells of ascending thick limb and distal tubules) and **other plasma proteins**.

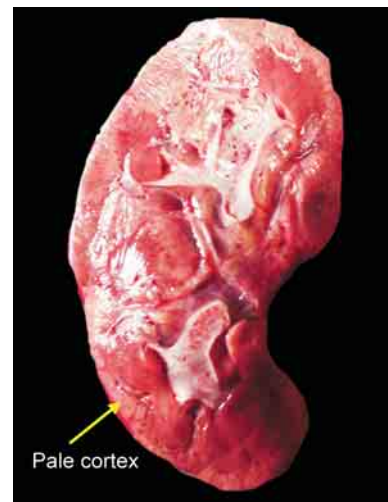
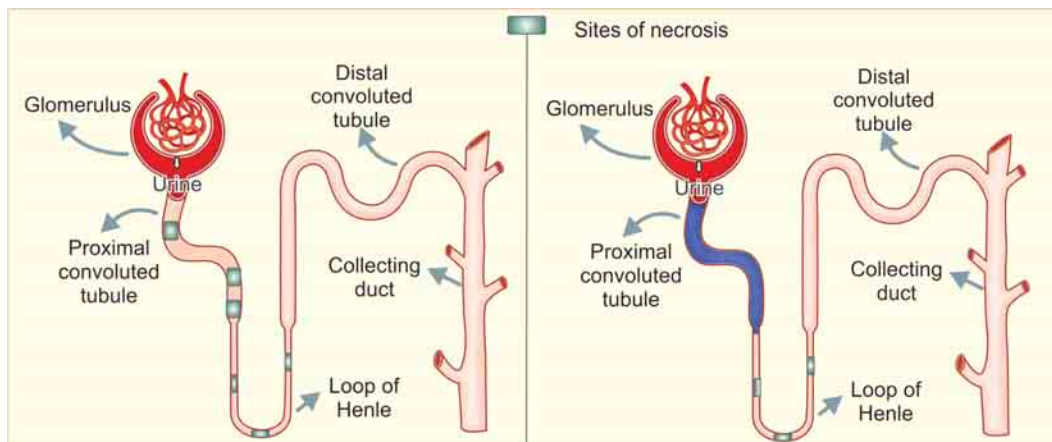


Fig. 21.34: Acute kidney injury. Gross appearance showing a pale cortex and a congested medulla

- Occlusion of tubular lumens:** The casts occlude the lumen and result in **dilation of the lumen**.
- Rupture of basement membranes** (tubulorrhexis): It can occur in later stages.
- Epithelial regeneration:** If the precipitating cause is removed, the tubular epithelium can regenerate and tubules may return to normal without any residual evidence of damage.
- Interstitial:** It shows edema and accumulations of leukocytes.
- Blood vessels:** Normal.

Tamm-Horsfall protein: Urinary glycoprotein normally secreted by the cells of ascending thick limb and distal tubules.



Figs 21.35A and B: Patterns of tubular damage in ischemic and toxic acute kidney injury; (A) In ischemic type, tubular necrosis is patchy, relatively short lengths of tubules are affected, and most marked in the straight segments of proximal tubules and ascending limbs of loop of Henle; (B) In toxic acute kidney injury, extensive necrosis involves the proximal convoluted tubule segments

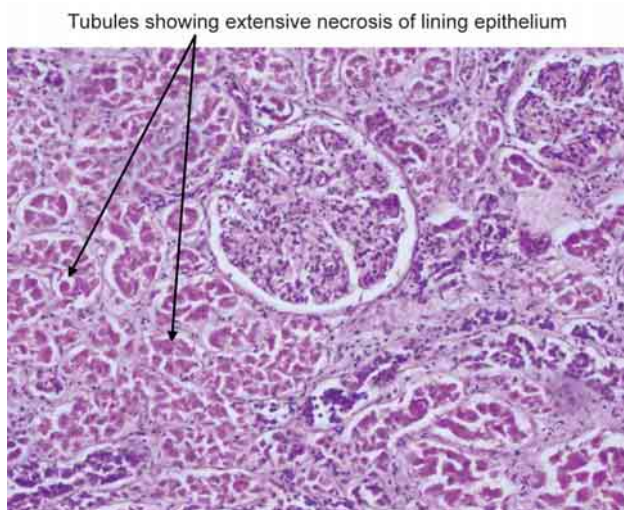


Fig. 21.36: Photomicrograph of acute tubular necrosis showing tubules with necrosis of tubular epithelial cells. The glomerulus appears normal

Toxic AKI

Microscopy

- Areas involved (Fig. 21.35B): Most **common site of toxic injury** is seen in the **proximal convoluted tubules**. The **necrosis of tubular epithelium is more-extensive** in toxic ATN than seen in ischemic ATN.
- Tubular necrosis is limited to certain segments of tubules that are most sensitive to the particular toxin.
- **ATN due to hemoglobin or myoglobin** show many **red-brown colored** (due to heme pigments) **tubular casts**.

Changes in tubular epithelial cells induced by few poisons and organic solvents are presented in Table 21.5.

TABLE 21.5: Tubular epithelial changes in direct tubular toxicity by poison and organic solvents

Poison/organic solvent	Changes in tubular epithelial cells
• Mercuric chloride	• Large acidophilic inclusions
• Carbon tetrachloride	• Accumulation of neutral lipids
• Ethylene glycol	• Ballooning and vacuolar degeneration of cells of proximal convoluted tubule
	• Calcium oxalate crystals in tubular lumen

Clinical Course

3 phases of acute kidney injury

- Initiation
- Maintenance
- Recovery.

The clinical course of AKI may be divided into three stages namely: (1) initiation, (2) maintenance, and (3) recovery phases.

1. Initiation Phase

Clinical features depend on the initiating cause of AKI. There is a mild reduction of urine output and increase in BUN.

2. Maintenance Phase

During this phase, there is sustained decrease in urine output in the range of 40 to 400 mL/day (oliguria), salt and water overload, rising BUN level, hyperkalemia, metabolic acidosis, and other features of uremia.

3. Recovery Phase

During this phase, there is a steady increase in urine volume, which may reach up to 3 L/day. There is loss of large amounts of water, sodium and potassium (leading to hypokalemia) in the urine. Once the renal tubular function returns to normal, BUN and creatinine levels also return to normal.

Prognosis: ATN is a **potentially reversible condition** and with modern therapy most patients recover.

Common causes of acute renal failure are shown in Table 21.6.

TABLE 21.6: Common causes of acute renal failure and associated urinary findings

Causes of acute renal failure	Urinalysis findings
Acute tubular necrosis	Dirty brown casts and epithelial cells
Acute glomerulonephritis	RBC casts and proteinuria
Acute tubulointerstitial nephritis	WBC casts and pyuria

URINARY TRACT OBSTRUCTION (OBSTRUCTIVE UROPATHY)

Urinary tract obstruction is caused by **structural or functional abnormalities** and results in **obstruction to the flow of the urine** (Fig. 21.37).

Consequences:

- Unrelieved obstruction almost always leads to:
 - Renal dysfunction (**obstructive nephropathy**) and permanent renal atrophy.
 - Dilation of the collecting system (hydronephrosis).
- Increased **susceptibility to infection** and to **stone formation**.

Hydronephrosis

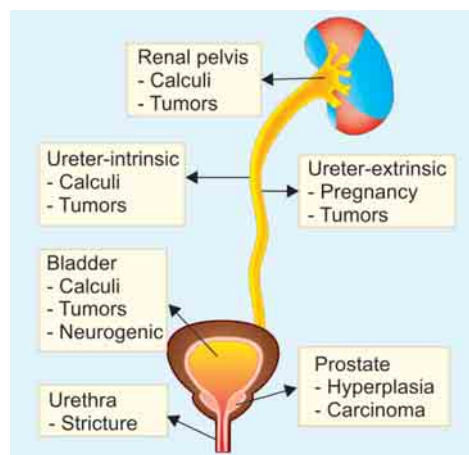
Q. Write short note on hydronephrosis.

Definition: Hydronephrosis is defined as an aseptic **dilation of the renal pelvis and calyces due to obstruction of urinary outflow**, associated with **progressive atrophy of the kidney**.

Hydronephrosis: Dilation of the renal pelvis and calyces due to obstruction of urinary outflow.

Causes (Fig. 21.37)

It may be classified as structural or functional disorders.



Hydronephrosis: Nature of obstruction may be:

- Congenital or acquired
- Sudden or insidious
- Partial or complete
- Unilateral or bilateral
- Intrinsic lesions or extrinsic lesions
- May occur at any level of the urinary tract from the urethra to the renal pelvis.

Fig. 21.37: Various common obstructive lesions of the urinary tract

1. Structural disorders:

- **Urinary calculi**
- **Tumors:** Carcinoma of the prostate, bladder tumors, carcinoma of the cervix or uterus
- **Benign prostatic hypertrophy**
- **Congenital anomalies:** Urethral strictures, meatal stenosis, bladder neck obstruction
- **Inflammation:** Prostatitis, urethritis, retroperitoneal fibrosis
- Pregnancy, uterine prolapse and cystocele.

2. Functional disorders:

- Neurogenic bladder (spinal cord damage or diabetic neuropathy).

Renal stones: Most common cause of upper urinary tract obstruction.

Pathogenesis

- **Obstruction:** It may be **complete or incomplete**. Obstruction in the urinary tract → leads to **accumulation of urine proximal to the obstruction**.
- **Dilatation:** Even with complete obstruction, **glomerular filtration** does not stop but **continues for some time** and → leads to **accumulation of urine** → causes **dilatation of affected calyces and pelvis** due to back pressure. Raised pressure in the renal pelvis → transmitted back through

the collecting ducts into the renal parenchyma and its consequences are:

- **Renal atrophy**
- **Compresses the renal vasculature of the medulla** → reduces the blood flow to the medulla with **diminished tubular function**.
- **Reduced GFR:** If obstruction persists, the functional alterations of tubule results in loss of concentrating function and later **reduces the glomerular filtration rate**.
- **Interstitial inflammation:** Obstruction also initiates an **interstitial inflammatory reaction** and **interstitial fibrosis**.

MORPHOLOGY

Hydronephrosis: Most common complication of upper urinary tract obstruction.

Type of Obstruction and its Consequence

- **Sudden and complete obstruction:** It reduces the glomerular filtration and leads to **mild dilation** of the pelvis and calyces.
- **Subtotal or intermittent obstruction:** It does not suppress glomerular filtration, and **produces progressive dilation**.

Level of Obstruction

Depending on the level of urinary obstruction, the dilation may first affect the bladder, or ureter and then the kidney.

Gross

- Depending on the level of obstruction, it may be **unilateral or bilateral** and may be accompanied by **dilatation of ureter (hydroureter)**.
- Depending on the degree and the duration of the obstruction, kidney may show **slight to massive enlargement**.
 - **Early stage:** Simple **dilation of the pelvis and calyces**
 - **Late stage:** Progressive **blunting of the apices of the pyramids**
 - **Advanced stage:**
 - ◆ **Kidney may appear like a thin-walled, cystic structure** having a diameter of up to 15 to 20 cm.
 - ◆ **Renal parenchyma shows destruction due to severe pressure atrophy and thinning of the cortex.** A kidney destroyed by longstanding hydronephrosis appear as a thin-walled, lobulated, fluid-filled sac (Fig. 21.38).

Microscopy

- **Tubules:** Dilatation and atrophy.
- **Glomeruli:** Partial or complete sclerosis.
- **Interstitium:** Marked chronic inflammatory infiltrate and fibrosis.

Clinical Features

- Clinical features **depend on the cause of obstruction**. For example, calculi in the ureters may present with renal colic, and prostatic enlargements may present with bladder symptoms.

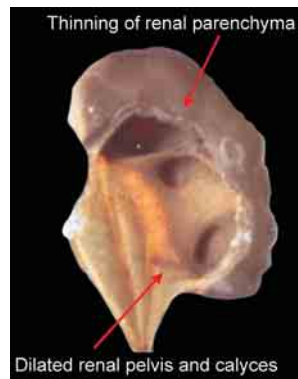


Fig. 21.38: Marked hydronephrosis of the kidney showing marked dilation of the pelvis and calyces and thinning of the renal parenchyma

Hydronephrosis: Pelvicalyceal dilatation causes atrophy and destruction of renal parenchyma.

- **Unilateral complete or partial hydronephrosis** may be silent due to maintenance of adequate renal function by the unaffected kidney.
- **Bilateral partial obstruction** may manifest as **polyuria and nocturia** due to inability to concentrate the urine (tubular dysfunction).
- **Complete bilateral obstruction** present with **oliguria or anuria**.
- **Ultrasonography** is a **useful** in the diagnosis of obstructive uropathy.

Urolithiasis (Renal Calculi, Stones)

Q. Write short note on renal stones.

Stones may be formed anywhere in the urinary tract, but most are found in the renal pelvis and calyces kidney.

- **Terminology**
 - **Nephrolithiasis (renal stones)**—stones within the collecting system of the kidney.
 - **Urolithiasis (urinary calculi/stones)**—stones anywhere in the collecting system of the urinary tract.
 - A primary bladder stone is one that develops in sterile urine; it often originates in the kidney.
 - A secondary stone occurs in the presence of infection, outflow obstruction, impaired bladder emptying or a foreign body.
- **Age:** Peak is between 20 and 30 years.
- **Sex:** More common in men than in women.

Renal stones: Most common cause of upper urinary tract hematuria.

Etiology

1. **Familial and hereditary predisposition** to stone formation is well-known.
 - Many inborn errors of metabolism (like gout, cystinuria, and primary hyperoxaluria) are characterized by

excessive production and excretion of stone-forming substances.

2. Other factors:

- Individual factors
- Geography
- **Diet:** Deficiency of vitamin A causes desquamation of epithelium and these cells may form a nidus on which a stone can be deposited.
- Metabolic alterations
- **Altered urinary solutes and colloids:** Dehydration increases the concentration of urinary solutes and are liable to precipitate.
- **Infection:** It favors the formation of calculi. Stone formation are common when urine is infected with urea-splitting streptococci, staphylococci and, especially *Proteus*.
- **Decreased urinary citrate:** Citrate in urine present as citric acid and is under hormonal control. It tends to keep otherwise relatively insoluble calcium phosphate and citrate in solution. Urinary excretion of citrate is decreased during menstruation.
- Changes in urinary pH.
- Urinary stasis: It favors stone formation.

Pathogenesis of Renal Stones

There are two main steps involved in stone formation: Initiation and propagation of stones.

1. **Supersaturation:** Initiation starts with the supersaturation of urine.
 - **Increased urinary concentration of stone constituents:** It is the most important factor in stone formation → exceeds their solubility (supersaturation).
 - **Decreased urine volume:** It may also favor supersaturation.
2. **Precipitation of crystals**
 - **Deficiency in inhibitors of crystal formation in urine enhances precipitation of crystals.** These inhibitors include: pyrophosphates, citrates, glycosaminoglycans, osteopontin, and a glycoprotein called **nephrocalcin**.
 - All calculi consist of an organic mucoprotein matrix, which makes up 1–5% of the stone by weight. The mucoproteins in the urine provide the organic nidus on which the crystals form.

General Features of Renal Stones

- Stones are **unilateral** in about 80% of patients.
- **Number:** It may be **single or multiple**.
- **Sites:** Renal calyces and pelvis and in the bladder.

BOX 21.8: Types and causes of renal stones

Type

1. **Calcium oxalate and/or calcium phosphate (~80%)**
 - Idiopathic hypercalciuria—most common
 - Hypercalciuria and hypercalcemia
 - Hyperoxaluria: Enteric, primary
 - Hyperuricosuria
 - Idiopathic
2. **Struvite (magnesium, ammonium, phosphate) (~10%)**
 - Urinary tract infection
3. **Uric acid (~7%)**
 - Associated with hyperuricemia
 - Associated with hyperuricosuria
 - Idiopathic
4. **Cystine (~2%)**
5. **Others/Unknown (~1%)**

Birefringent crystals in urine is seen in: Calcium oxalate stones.

- **Shape:** Stones may have **smooth contours** or may be **irregular, jagged mass of spicules**.

Types of Renal Stones (Box 21.8)

There are **four main types** of calculi:

1. **Calcium stones (~80%):** Composed of calcium oxalate or calcium phosphate or mixture of both.
2. **Triple stones or struvite stones (~10%):** Composed of magnesium, ammonium and phosphate.
3. **Uric acid stones (~7%).**
4. **Cystine stones (~2%).**

1. Calcium Stones (Oxalate Calculus/Calcium Oxalate)

- Most (80%) renal stones are **composed of calcium complexed with oxalate** (calcium oxalate) or **phosphate** (calcium phosphate) or a mixture of these (calcium oxalate + calcium phosphate). These stones are radiopaque.

Etiology (Box 21.8)

Renal stones: Calcium stones are most common.

Calcium renal stones: Most common metabolic cause is hypercalciuria.

- **Hypercalciuria without hypercalcemia:** It is observed in about 50% of patients. Causes include: Hyperabsorption of calcium from the intestine (absorptive hypercalciuria), an intrinsic impairment in renal tubular reabsorption of calcium (renal hypercalciuria) or idiopathic.

- **Hypercalcemia and hypercalciuria:** It is found in about 10% of patients and may be due to hyperparathyroidism, diffuse bone disease, or sarcoidosis.
- **Hyperuricosuria:** It causes “nucleation” of calcium oxalate in the collecting ducts.
- **Hyperoxaluria:** It may be either hereditary (primary oxaluria) or acquired.
- **Idiopathic:** These stones develop without any identifiable cause.

MORPHOLOGY

Calcium oxalate:

- Needle-shaped/square envelope-shaped
- Radiopaque.
- **Calcium oxalate stone:** It is irregular in shape **hard** and covered with **sharp projections**. **Hemorrhage** from the mucosa of the renal pelvis may be produced by its sharp edges and blood may cover the stone **making it to appear black**. It is radiodense.
- **Calcium phosphate stone:** Soft and pale.

2. Struvite Stones or (Triple stones/ Magnesium, Ammonium, Phosphate Stones)

They are composed of calcium phosphate often with magnesium and ammonium phosphate, and are **known as struvite stones or triple phosphate stones**.

Etiology

Struvite stones: Develop after urinary tract infections by urea-splitting bacteria (e.g. *Proteus*).

- They **develop after infections of the urinary tract by urea-splitting bacteria** (e.g. *Proteus*), which convert urea to ammonia → produces alkaline pH + slowing of urine flow → precipitation of magnesium, ammonium, phosphate (struvite), and calcium phosphate (apatite).

MORPHOLOGY

Struvite (triple) stone:

- Coffin lid shaped
- Radiolucent.
- **Yellow-white and solitary**
- **Hard to soft and friable**
- **Largest stones**, because normally large amounts of urea is excreted
- **Sometimes fill the pelvis and calyces to form a cast** of these spaces → referred to as a **staghorn calculus** (Fig. 21.39)
- Even a very large staghorn calculus may be clinically silent for years until it produces hematuria, urinary infection or renal failure
- Easily seen on radiographic films.

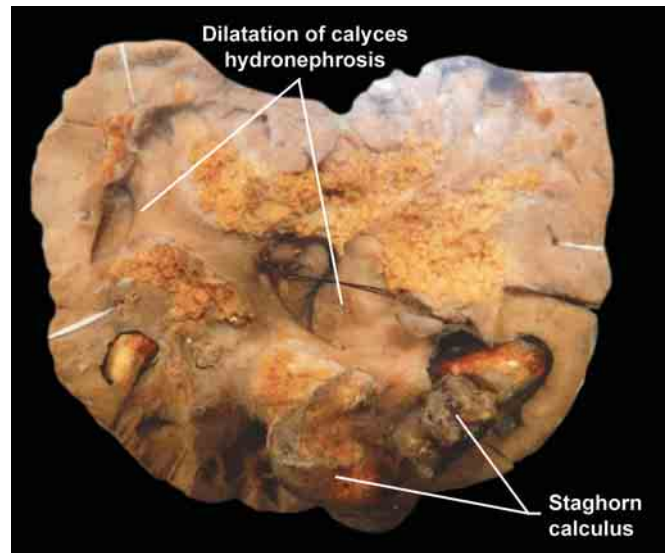


Fig. 21.39: Cut opened kidney showing staghorn calculus forming the cast of calyces

Complications: Intractable urinary tract infection, pain, bleeding, and perinephric abscess.

3. Uric Acid and Urate Stones

Etiology

- Commonly found in patients with **hyperuricemia** (e.g. gout) and **diseases involving rapid cell turnover** (e.g. leukemias).
- However, more than 50% of patients have neither hyperuricemia nor increased urinary excretion of uric acid.
- **Uric acid is insoluble in acidic urine and urine pH below 5.5 may predispose to uric acid stones.**

MORPHOLOGY

Uric acid:

- Diamond/barrel shape
- Radiolucent.
- Uric acid stones are **radiolucent**; this is in contrast calcium stones, which are radiopaque.
- **Smooth, hard, and** vary from yellow to reddish-brown. Sometimes have multifaceted appearance.
- Usually **multiple** and are **less than 2 cm** in diameter.
- Show **lamination on cut section**.

Renal stones: 80% are radiopaque.

4. Cystine Stones

Cystine stone:

- Hexagonal
- Radiopaque.

Etiology

- Cystine stones are uncommon and associated with **cystinuria**, which is due to genetic defects in the renal reabsorption of cystine or other amino acids.
- Stones form at **low urinary pH** (acidic urine).

MORPHOLOGY

- Cystine stones are **small, round, smooth** and usually **multiple**
- **Yellow and waxy.**
- They are very hard and radiopaque because of their sulfur content.

Clinical Features of Renal Stones

Renal stones: Ultrasound can detect only hydronephrosis and not the renal stone.

- Stones may be **asymptomatic** or **may obstruct urinary flow or produce ulceration and bleeding.**
- Small stones **may pass into the ureters**, producing colic and ureteral obstruction.
- **Larger stones** cannot enter the ureters and likely to **remain silent** within the renal pelvis. Larger stones may present with **hematuria**.
- Stones also **predispose to superimposed infection** and may also **cause significant renal damage.**

Complications of Renal Stones

- **Hematuria**
- **Hydronephrosis** due to obstruction
- **Pyelonephritis and pyonephrosis**
- **Carcinoma:** Stones can cause squamous metaplasia and later squamous cell carcinoma.

MALIGNANT TUMORS OF THE KIDNEY

Both benign and malignant tumors can occur in the kidney. Most common malignant tumors are renal cell carcinoma and Wilms tumor. WHO classification (abridged) of tumors of the kidney is presented in Box 21.9.

Q. Classify tumors of kidney.

Renal Cell Carcinoma (Adenocarcinoma of the Kidney, Hypernephroma, Grawitz Tumor)

Q. Write short note on renal cell carcinoma.

Renal cell carcinoma (RCC) is a **malignant tumor** of kidney.

- **Cell of origin:** The tumors arise from renal tubular epithelium.

BOX 21.9: WHO classification of tumors of the kidney

Renal cell tumors

- Clear cell renal cell carcinoma
- Papillary renal cell carcinoma
- Chromophobe renal cell carcinoma
- Carcinoma of the collecting ducts of Bellini
- Papillary adenoma
- Oncocytoma

Nephroblastic tumors

- Nephroblastoma (Wilms tumor)

Metanephric tumors

- Metanephric adenoma

Mesenchymal tumors

- Occurring mainly in children
 - Clear cell sarcoma
 - Rhabdoid tumor
- Occurring mainly in adults
 - Leiomyosarcoma (including renal vein)
 - Angiosarcoma
 - Rhabdomyosarcoma

Others

Metastatic tumors

- Because of their **gross yellow color** and the **microscopic resemblance of the tumor cells to clear cells of the adrenal cortex**; these tumors were **originally** thought to arise from embryonic adrenal rests and **were called hypernephroma.**

Most common type of RCC is sporadic.

- **Age:** Usually in the **sixth and seventh decades** of life.

Most common histological type of RCC clear cell carcinoma.

- **Sex:** **More common in males** with male to female ratio of 2:1.

Etiology

Risk Factors

- **Tobacco:** Cigarette and pipe smoking
- Obesity (mainly in women)
- Hypertension
- Unopposed estrogen therapy
- Exposure to asbestos, petroleum products, and heavy metals
- Chronic renal failure and acquired cystic disease
- Tuberous sclerosis.

Most common type of RCC seen with dialysis associated cystic disease: Papillary carcinoma.

Types

- **Sporadic:** Most (96%) of RCC are sporadic.
- **Hereditary/inherited/familial:** About 4% of RCC are inherited. All hereditary RCC are **multifocal** and **bilateral**, and occur at a younger age than sporadic RCC. It occurs in three distinct syndromes.
 1. **Von Hippel-Lindau (VHL) syndrome:** *VHL* gene is a tumor suppressor gene and is involved in the development of **both familial and sporadic clear cell carcinoma**. VHL patients develop bilateral renal cysts and multiple renal cell carcinomas.

VHL syndrome: Autosomal dominant cancer syndrome, characterized by:

- Cerebellar hemangioblastomas
- Retinal angiomas
- Clear cell RCC
- Pheochromocytoma
- Cysts in various organs.

2. **Hereditary leiomyomatosis and renal cell cancer syndrome:** It is an autosomal dominant disease due to mutations of the *FH* gene (expresses fumarate hydratase). It is characterized by leiomyomata (cutaneous and uterine) and an aggressive type of papillary carcinoma with increased tendency for metastatic spread.
3. **Hereditary papillary carcinoma:** It is **autosomal dominant** form with **multiple bilateral tumors** showing **papillary growth pattern**. These tumors show a several cytogenetic abnormalities and **mutations in the *MET* proto-oncogene**.
4. **Birt-Hogg-Dubé syndrome:** It is an autosomal dominant disease is due to mutations of the *BHD* gene (which expresses folliculin). It is characterized by lesions of skin (fibrofolliculomas, trichodiscomas, and acrochordons), lung (cysts or blebs), and renal tumors of various histological subtypes.

Triad of VHL syndrome:

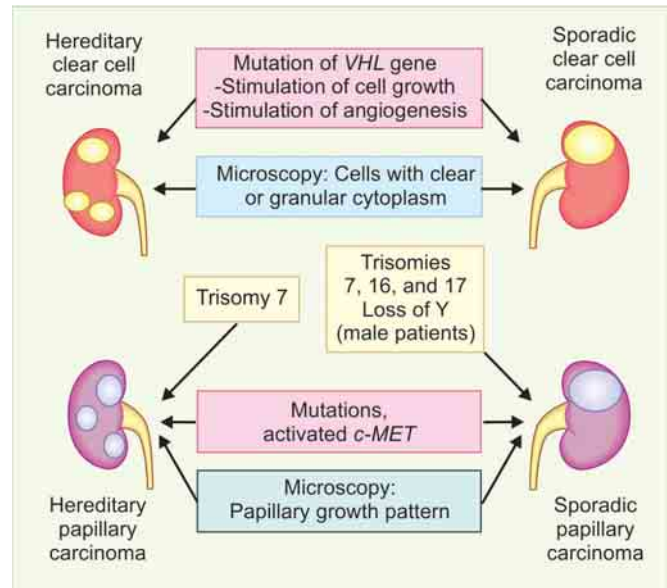
1. Cerebellar hemangioblastoma
2. Bilateral renal cell carcinoma
3. Retinal angiomas.

Classification of Renal Cell Carcinoma (Fig. 21.40)

Both sporadic and familial forms of renal cell carcinoma can be classified depending on the cytogenetics, genetics, and microscopic features into four major types.

1. Clear cell carcinoma

Clear cell carcinoma: Associated with homozygous loss of tumor suppressor gene *VHL*. *VHL* gene is localized to 3p.



Both sporadic and familial RCC are associated with: Loss of sequence on chromosome 3 either by translocation (3:6, 3:8, 3:11) or deletion. This region harbors the *VHL* gene.

Familial RCC is associated with: Von Hippel-Lindau syndrome.

Fig. 21.40: Cytogenetics, genetics and microscopic features of clear cell and papillary renal cell carcinoma

- **Most common type** (~70%).
- **Most (95%) are sporadic;** but they can be familial.
- **Cell of origin:** Arise from **proximal tubular epithelial cells**.
- Usually occur as **solitary** and **unilateral lesions**.
- **Genetic mechanism:** *VHL* gene is localized to 3p, and acts as a tumor suppressor gene in both sporadic and familial forms of cancers. **Effects of loss or mutation of *VHL* gene are:**
 - **Stimulation of cell growth**
 - **Stimulation of angiogenesis:** *VHL* gene encodes a protein that degrades other proteins like hypoxia-inducible factor-1 (HIF-1).
 - ♦ Mutated *VHL* → HIF-1 is not degraded → HIF-1 levels remain high → increases the production of proangiogenic proteins such as VEGF, PDGF, TGF- α , and TGF- β → stimulates angiogenesis.

2. Papillary carcinoma

Papillary carcinoma of kidney: Most common cytogenetic abnormalities are trisomies 7, 16, and 17.

- ~10–15% of renal carcinomas.
- Occurs in both **familial** and **sporadic** forms (Fig. 21.41).

- **Cell of origin:** Arise from **distal convoluted tubule epithelial cells**.
- Frequently **multifocal** and **can be bilateral**.
- **Hereditary papillary RCC is not associated with deletions of VHL gene.**
- **Mutations in the c-MET, a proto-oncogene (MET) is found in both the sporadic and familial forms.**
- **Cytogenetic abnormalities:**
 - **Sporadic form: (1) trisomies 7, 16, and 17, and (2) loss of Y in male patients**
 - **Familial/hereditary form: Trisomy 7.**

Papillary carcinoma of kidney: Trisomy in familial form due to activating mutations of MET oncogene on chromosome 7.

3. Chromophobe renal carcinoma

RCC associated with best prognosis: Chromophobe carcinoma.

- ~5% of renal cell carcinomas
 - **Cytogenetic abnormalities:** Multiple chromosome losses and extreme hypodiploidy
 - **Cell of origin:** Arise from **intercalated cells of renal collecting ducts**
 - **Excellent prognosis** compared to clear cell and papillary cancers.
4. **Collecting duct (Bellini duct) carcinoma**
- ~ 1% or less of renal carcinoma
 - **Cell of origin:** Arise from the **medullary collecting duct and occur in the medullary region**. Medullary carcinoma is a morphologically similar tumor developing in patients of sickle cell trait.
 - **Cytogenetic abnormalities:** **No distinct pattern** but several chromosomal losses and deletions have been described.
5. **Xp11 translocation carcinoma:** It is a genetically distinct subtype of renal cell carcinoma that develops in young individuals. It is associated with translocations of the TFE3 gene located at Xp11.2 with a number of partner genes that lead to overexpression of the TFE3 transcription factor. The tumor cells have clear cytoplasm and papillary architecture.

MORPHOLOGY

Renal cell carcinoma: Yellow tumor with variegated appearance.

Q. Write short note on morphology of renal cell carcinoma.

Gross

A. General features of RCC:

- **Site:** May arise in **any part** of the kidney. More commonly affects the poles, **mostly upper pole**
- **Size varies**

- **Outer surface:** It is usually **bosselated** and may distort the renal outline.
- B. **Cut surface (Fig. 21.41)**
- Tumor is **solid, bright yellow-gray-white** and shows **areas of hemorrhage, necrosis and cystic change**, which give a **variegated appearance**. The **yellow color** is due to **prominent lipid** in the tumor cells.
 - Tumor may **appear circumscribed, spherical masses with well-demarcated margins**. But may show some breach in the renal capsule and invasion into the perinephric fat.

RCC: Yellow color is due to lipid in the tumor cells.

C. Venous invasion:

Renal cell carcinoma: Tendency to invade renal vein.

- **One of the characteristics of RCC** is its **tendency to invade the renal vein**.
- Sometimes the tumor may grow as a continuous cord in the inferior vena cava and rarely may extend into the right atrium.

Microscopy

1. Clear cell carcinoma (Fig. 21.42)

- **Growth pattern:** Varies from **solid to trabecular (cord-like) or tubular** (resembling tubules or glands).
- **Tumor cells:** Tumors are **composed of cells with clear or granular cytoplasm** and are **nonpapillary**.
 - **Shape:** The tumor cells are **round or polygonal** in shape.
 - **Cytoplasm:**
 - ◆ **Abundant** either **clear or granular**.
 - ◆ **Clear cells** have sharply outlined cell membrane ("vegetable cells"). The clear cytoplasm is due to **glycogen and lipids**; which are removed by the water and solvents (xylene) used during processing of tissue for histopathological examination.
 - ◆ **Special stains:** **Glycogen may be demonstrated with PAS and fat by oil red O stains.**
 - ◆ **Nucleus:** It is **centrally located** and **small**, which falsely mask the malignant nature of this tumor.
- **Stroma:** **Consists of branching vasculature.**
- **Most tumors** are well-differentiated with **little cellular or nuclear pleomorphism**.

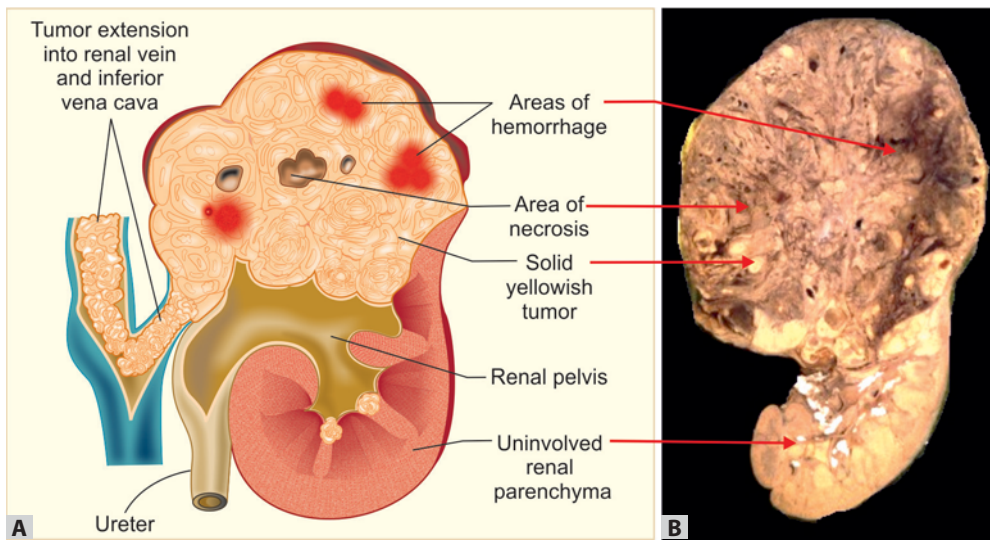
Clear cell RCC: Nucleus has raisin appearance.

Notable features of RCC:

1. Encapsulated in spite of being malignant (pseudocapsule)
2. Spontaneous regression
3. Prolonged period of stable disease
4. Refractoriness to cytotoxic agents.

2. Papillary tumors (Fig. 21.43A): It shows a papillary growth pattern.

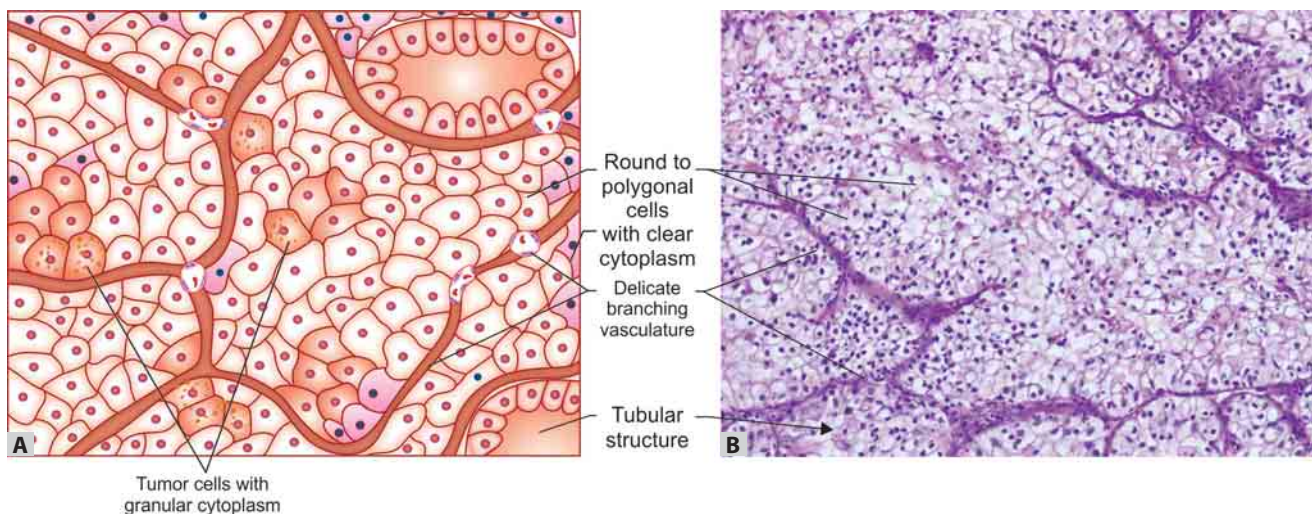
- **Papillary configuration:** Tumor is composed of cuboidal or low columnar tumor cells arranged in papillary formations. Interstitial foam cells are common in the fibrovascular stalks/cores of papillary projections.



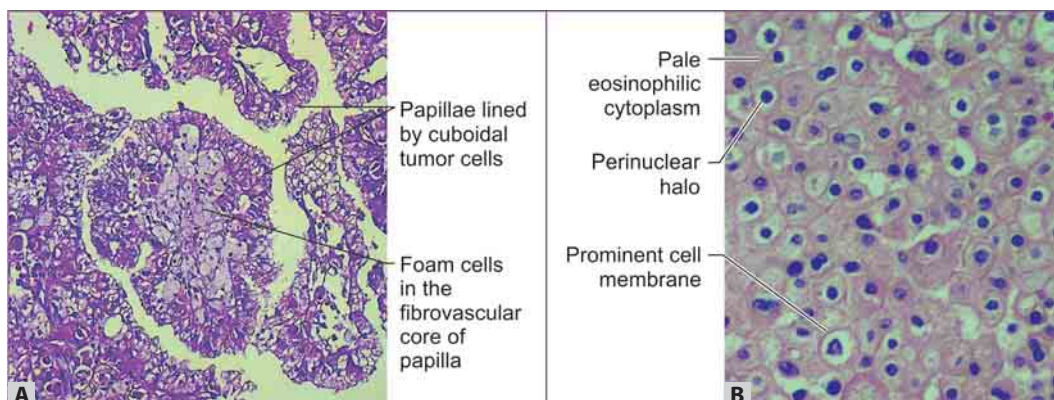
Papillary carcinoma of the kidney:

- Strong tendency to invade the renal vein and grow as solid column of cells.
- Tumor may even extend to the inferior vena cava and right side of the heart.

Figs 21.41A and B: Cut surface of renal cell carcinoma showing a yellowish, spherical, circumscribed, variegated tumor at the upper pole of kidney. (A) (Diagrammatic); (B) Gross specimen



Figs 21.42A and B: Renal cell carcinoma. (A) Diagrammatic; (B) Photomicrograph of clear cell type showing solid groups of clear cells separated by vascular stroma



Figs 21.43A and B: (A) Papillary carcinoma showing papillae lined by cuboidal tumor cells and fibrovascular core with foam cell; (B) Chromophobe renal carcinoma showing tumor cells with prominent membrane, pale eosinophilic cytoplasm and perinuclear halo

- Cytoplasm may be eosinophilic or basophilic.
 - Psammoma bodies may be seen.
 - Stroma is usually scanty and highly vascularized.
- 3. **Chromophobe renal carcinoma (Fig. 21.43B):** It consists of **granular cells arranged in solid sheets** with a concentration of the largest cells around blood vessels. The tumor cells have:
 - Prominent cell membranes
 - Pale eosinophilic cytoplasm
 - A halo around the nucleus (perinuclear halo).
- 4. **Collecting duct carcinoma:** It consists of **nests of malignant cells separated by prominent fibrotic stroma**. It shows **irregular channels lined by highly atypical epithelium with a hobnail pattern**.

Sarcomatoid changes develop infrequently in all types of renal cell carcinoma and are of poor prognostic feature.

Clinical Features

Clinical features of RCC: Triad of

1. Flank pain
2. Abdominal mass
3. Hematuria

Classical triad is seen in only 10% of cases.

Classical diagnostic triad (seen in only 10% of cases) of renal cell carcinoma are:

- **Costovertebral or flank pain**
- **Palpable abdominal mass**
- **Hematuria (most reliable).**

Renal cell carcinoma **can produce ectopic hormones and paraneoplastic syndromes** (Table 21.7).

Renal cell carcinoma: Paraneoplastic syndrome with ectopic production of erythropoietin and PTH-related peptide.

Erythropoietin: Major source is interstitial cells in peritubular capillaries and tubular epithelial cells.

TABLE 21.7: Paraneoplastic syndromes produced by renal cell carcinoma

• Polycythemia (due to erythropoietin)	• Hepatic dysfunction (due to IL-6 leading to increased ALP, PT and bilirubin)
• Hypercalcemia (due to PTH-rp)	• Feminization or masculinization
• Hypertension (due to renin)	• Cushing's syndrome

Canon ball pulmonary metastases: Characteristic features of

1. RCC (produces spherical or round cannon-ball metastases)
2. Testicular cancer.

Spread

Patients may **develop wide metastasis** before giving rise to any local symptoms or signs.

- **Local spread:** It invades perinephric fat.
- **Lymphatic spread:** It spreads to regional lymph nodes occurs when tumor extends beyond renal capsule.
- **Hematogenous spread:** Most common route. Most common sites are lungs (cannon ball deposits and pulsating secondaries) > bones > liver > adrenal > brain.

Prognosis: Depends on the extent of tumor.

Wilms Tumor (Nephroblastoma)

Wilms tumor: Most common primary kidney tumor in children (2 to 5 years). Second most common malignant abdominal tumor in children.

- Wilms tumor is **most common primary renal tumor of childhood**.
- **Highly malignant primary embryonal tumor.**
- **Age group:** Most common between **2 and 5 years of age**, and more than 95% occur below 10 years of age.

Pathogenesis and Genetics

WT1 gene: Tumor suppressor gene located in the chromosome 11p13.

Wilm tumor: Deletion of short arm of chromosome 11p13.

- In **most (90%)** cases, the Wilms tumor is **sporadic and unilateral**.
- About 5–10% are bilateral which involve either simultaneously (synchronous) or one after the other (metachronous).
- **Mutation in tumor suppressor genes** is associated with Wilms tumor. These include **Wilms tumor associated genes 1 (WT1) and WT2**. **WT1 gene is located in the chromosome 11p13**.
- In **about 5% of cases**, Wilms tumor **arises in three congenital syndromes** at an early age and often bilaterally. These syndromes are:

Wilms' tumor: Associated congenital syndromes

1. WAGR syndrome
2. Denys-Drash syndrome
3. Blackwith-Wiedmann syndrome.

1. **WAGR** (for Wilms tumor, aniridia, genital anomalies, and mental retardation) **syndrome:** It has germline deletions of 11p13, region where Wilms tumor-associated gene (*WT1*) is located.

2. **Denys-Drash syndrome** (Wilms tumor, intersexual disorders, glomerulopathy) is associated with mutations of the *WT1* gene.
 3. **Beckwith-Wiedemann syndrome (BWS)** is associated with Wilms tumor and *WT2* gene imprinting abnormalities.
- Mutations of the β -*catenin* gene were found in few sporadic cases of Wilms tumors.

WT1 and PAX6 genes are located at chromosome 11p13.

- **Nephrogenic rests** (small foci of persistent primitive blastemal cells, which are precursor lesions of Wilms tumors) are seen in the renal parenchyma adjacent to bilateral Wilms tumors.
- Wilms tumor was **also found in association with other malignancies** (e.g. osteosarcoma, retinoblastoma, hepatocellular carcinoma and neuroblastoma).

MORPHOLOGY

Q. Write short note on morphology of Wilms tumor.

Gross (Fig. 21.44)

- Wilms tumor is **usually large, single, round, well-circumscribed mass**.
- **Usually unilateral** but 10% is either bilateral or multicentric.
- **Cut section:**
 - Tumor is **soft, bulging, homogeneous, and tan to gray**.
 - Foci of hemorrhage, cyst formation, and necrosis may be seen.

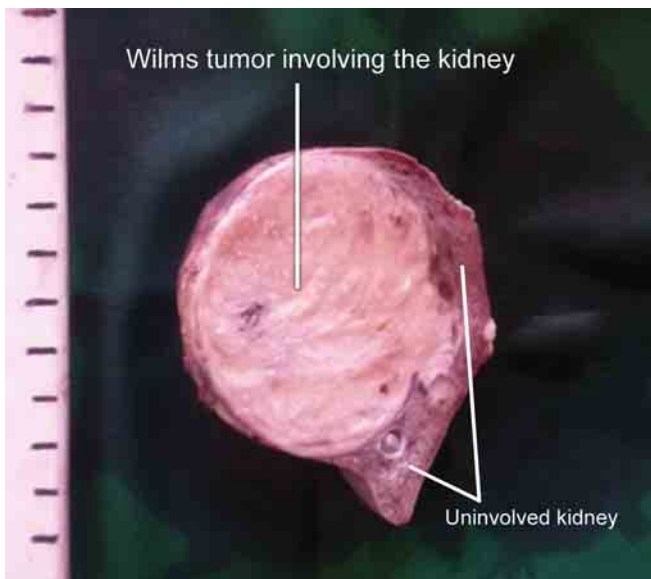


Fig. 21.44: Wilms tumor of the kidney with well-circumscribed margins

Microscopy (Fig. 21.45)

Wilms tumor microscopy: Three components

1. Blastemal
2. Immature stromal
3. Immature epithelial.

- Tumor shows **three major components**, which resemble normal fetal tissue. These cells attempt to recapitulate different stages of nephrogenesis.
- The **three types of cells** are:
 1. **Blastemal component:** It consists of **small, round to oval blue cells with scanty cytoplasm**. These cells are arranged in sheets, nests and trabeculae.
 2. **Immature stromal (mesenchymal) component:** It consists of **undifferentiated fibroblast-like spindle cells**. They may show **smooth muscle, skeletal muscle or fibroblast differentiation**.
 3. **Immature epithelial component:** Epithelial cells show **differentiation in the form of small abortive (embryonic) tubules or immature glomeruli**.

Classically, the tumor shows **triphasic** (all three cell types) combination, although the percentage of each component varies. Occasionally they contain only two elements (biphasic) or even only one (monophasic).

- **Anaplasia:** It is defined as the **presence of cells with large, hyperchromatic, pleomorphic nuclei and atypical mitotic figures**. Anaplasia is **associated with TP53 mutations** and such tumors **do not respond to chemotherapy**.

Anaplasia in Wilms tumor: Associated with

- *TP53* mutations
- Resistance to chemotherapy.

Clinical Features

Wilms tumor:

1. Abdominal mass
2. Microscopic hematuria.

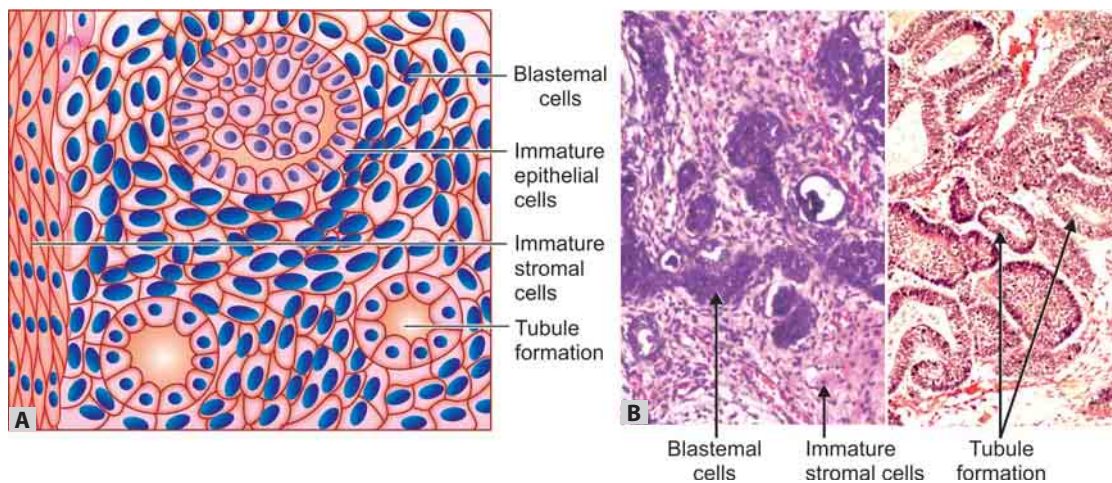
- Most children present with an **abdominal mass**, when large it may extend across the midline and down into the pelvis.
- Others: **Hematuria, pain in the abdomen**, intestinal obstruction, and pulmonary metastases are other patterns of presentation.

Spread

- **Local spread:** It spreads to perirenal soft tissues.
- **Lymphatics:** It spreads to regional lymph nodes.
- **Hematogenous:** Lungs, liver and peritoneum.

Prognosis

- **Clinical parameter:** Children **younger than 2 years of age** have a **better prognosis**.



Figs 21.45A and B: (A) Diagrammatic; (B) Hematoxylin and eosin (H & E) Wilms tumor shows highly cellular areas composed of tightly packed blue cells (undifferentiated blastema) separated by loose stroma containing undifferentiated mesenchymal cells, and immature (primitive) tubules

- **Histological parameter:**
 - **Invasion of the renal capsule** is associated with **poor prognosis**.
 - **Anaplasia** indicates a **poorer prognosis**.

UROTHELIAL TUMORS

Q. Write short note on transitional cell carcinoma of urinary bladder.

Transitional cell carcinoma: Most common tumor of urinary bladder.

- **About 95% of bladder tumors are of epithelial origin.** Most of the epithelial tumors of the bladder are composed of urothelial (transitional) cell type and are known as **urothelial or transitional tumors**.
- Urothelial tumors form about 90% of all bladder tumors. These tumors may range from small **benign lesions** to **aggressive cancers**.
- Many of urothelial tumors are **multifocal** and are most commonly seen in the **bladder**. But they may develop at any site where there is urothelium, from the renal pelvis to the distal urethra.

Precursor Lesions

Two precursor lesions of invasive urothelial carcinoma are:

1. **Noninvasive papillary tumors:** They show a range of atypical changes in the urothelial cells, and are graded according to their biological behavior.
2. **Flat noninvasive urothelial carcinoma:** It is known as **carcinoma in situ** or **CIS** and is characterized by cytologic changes of malignancy.

Epidemiology

- **More common in developed** than in developing countries, and in **urban** than in rural dwellers.
- These tumors are usually **not familial**.
- **Sex:** Higher in **males** than in females (male-to-female ratio is 3: 1).
- **Age:** Most between **50–80 years** of age.

Pathogenesis

A. Risk factors of urothelial carcinoma

Risk factors for urothelial carcinoma:

1. Cigarette smoking
2. Aromatic amines and azo dyes
3. Schistosoma hematobium
4. Analgesics
5. Cyclophosphamide
6. Radiation.

1. **Cigarette smoking:** It is the **most important risk factor** and risk depends on the amount of smoking and smoking habits. Most are associated with the use of cigarettes. Cigars, pipes and smokeless tobacco are associated with a minor risk.
2. **Industrial exposure to arylamines:** The **aromatic amines** (β -naphthylamine) and **azo dyes** that were widely used in the past in the **aniline dye and rubber industries** and are associated with bladder carcinoma. The cancers develop 15 to 40 years after the first exposure.

Mechanism of action:

- Both aromatic amines and azo dyes are metabolized in the liver.

- The aromatic amines are converted to active carcinogenic metabolite in the liver, which can be detoxified by conjugation with glucuronic acid.
 - The conjugated metabolite is excreted in the urine and deconjugated in the urinary tract by the enzyme glucuronidase, thus exposing the urothelium to the active carcinogen (reactive hydroxylamine).
3. **Schistosoma hematobium** infection: It is a risk factor in endemic areas (Egypt, Sudan). More than three-fourth of the cancers are **squamous cell type**, and remaining urothelial type.
 4. **Analgesics**: Long-term use of analgesics is a risk factor.
 5. **Cyclophosphamide**: It produces hemorrhagic cystitis, and increases the risk of carcinoma of the bladder.
 6. **Radiation**: Previous exposure of the bladder to irradiation (for other pelvic malignancies).
- B. **Genetic alterations in urothelial carcinoma**:
- **Chromosome 9 monosomy or deletions**: It frequently occurs in superficial papillary tumors and rarely in noninvasive flat tumors. The 9p deletions (9p21) involve 2 tumor suppressor genes: *p16 (INK4a)* and *p15*.
 - **Mutations in *p53*** are found in CIS and urothelial carcinoma.

MORPHOLOGY (BOX 21.10)

Site: Most urothelial tumors arise from the **lateral or posterior walls at the bladder base**.

Number: **Single or multicentric** producing separate tumors.

Gross (Fig. 21.46)

Carcinoma of urinary bladder: Most common

- Gross type is papillary
- Histological type is transitional cell carcinoma.

1. **Purely papillary**: These tumors appear as **red, elevated excrescences**. Size varies from small (less than 1 cm in diameter) to large masses (up to 5 cm in diameter). Majority of papillary tumors are low grade.
2. **Nodular**.
3. **Flat**.

Microscopy

They range from benign papilloma to highly aggressive anaplastic cancers.

1. **Urothelial papilloma**: It is uncommon and consists of two histological forms: Classical exophytic papilloma and inverted papilloma.

- **Exophytic papillomas**: They appear as a **delicate, finger-like papillary structures**. They have a central **core of loose fibrovascular tissue** covered by **transitional epithelium** that is microscopically **identical to normal urothelium**.
 - **Inverted papillomas**: These are rare and appear as nodular lesion in the mucosa of the urinary bladder, usually in the trigone area. They consists of **invagination of inter-anastomosing cords of normal transitional epithelium, down into the lamina propria**.
2. **Papillary urothelial neoplasms of low malignant potential (PUNLMPs)**
 - **Papillae**: They consist of papillae with a **fibrovascular core** covered by urothelium. The urothelium is **thicker than seen in papilloma**.
 - **Cytological features**: Urothelium with **uniform nuclear enlargement**. Mitotic figures are rare.
 - **May recur**.
 3. **Low-grade papillary urothelial carcinomas (Fig. 21.47)**
 - **Papillae**: It consists of **fused, branching and delicate papillae**. The fusion of papillae is focal.
 - **Cytological features**:
 - Papillae are lined by **neoplastic transitional epithelium** with **orderly appearance**, both architecturally and cytologically.
 - **Polarity is maintained**.
 - **Minimal nuclear atypia**: Mild variation in nuclear size and shape (**nuclear pleomorphism**), **scattered hyperchromatic nuclei** and **occasional mitotic figures may be seen**, mainly toward the base.
 - **Can recur**.
 4. **High-grade papillary urothelial carcinoma (Fig. 21.48)**
 - **Papillae**: It consists of **fused, branching and delicate papillae**.
 - **Cytological features**:
 - **Loss of polarity**: Architecturally, the epithelium is disorganized and consists of discohesive cells with frequent loss of polarity. Some of the tumor cells show frank anaplasia.
 - **Moderate to severe nuclear atypia**.
 - **Significant variation** in nuclear size and shape (**moderate to marked nuclear pleomorphism**).
 - **Significant nuclear hyperchromasia**.
 - **Frequent mitotic figures** in all layers, including atypical mitotic figures.
 - **Invasion**: About 80% of high grade urothelial carcinomas show invasion into the lamina propria, muscular layer or entire thickness of the bladder wall.
 - **Metastasis**: About 40% of invasive tumors may metastasize.
 - Regional lymph nodes
 - Hematogenous dissemination to liver, lungs, and bone marrow.

Carcinoma of bladder: Most common lymph node involved is obturator.

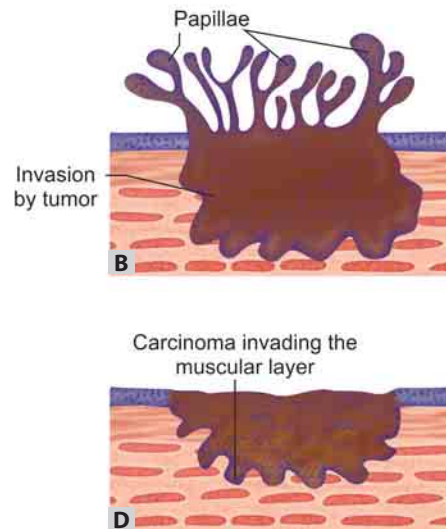
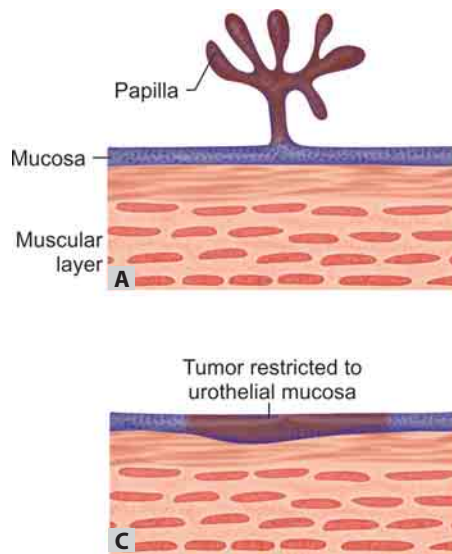
BOX 21.10: Grading of urothelial (transitional) tumors of the urinary bladder

1. Papilloma
 - Exophytic papilloma
 - Inverted papilloma
2. Papillary urothelial neoplasms of low malignant potential
3. Low grade and high grade papillary urothelial cancers
4. Carcinoma in situ (CIS, or flat non-invasive urothelial carcinoma)

Clinical Course of Bladder Cancer

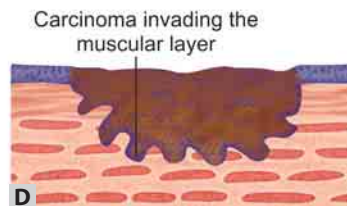
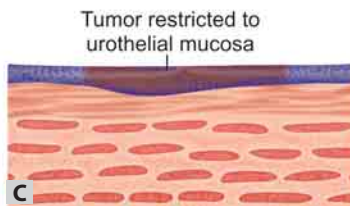
Transitional cell carcinoma of bladder: Most common non-infectious cause of lower urinary tract painless hematuria.

- **Painless hematuria:** Sometimes, it may be the only clinical feature. Sometimes the hematuria may be associated with frequency, urgency and dysuria.
- **Complications:** When the tumor obstructs the ureteral orifice, it may lead to pyelonephritis or hydronephrosis.



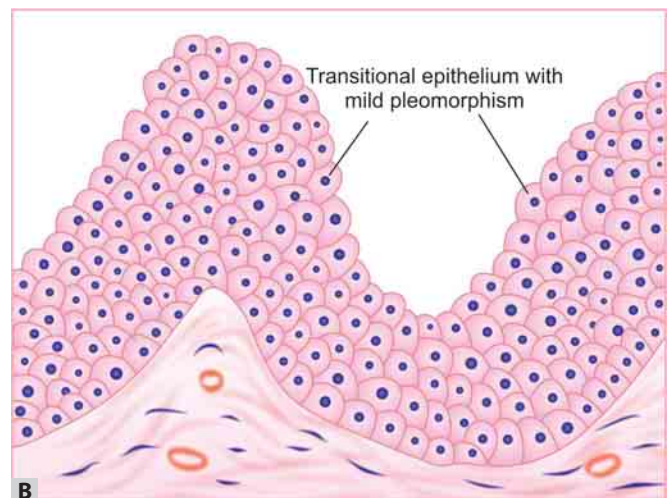
Bladder cancer is **NOT** caused by TB.

TCC of bladder: Involvement of detrusor muscle is associated with worst prognosis.



Figs 21.46A to D: Four morphologic patterns of tumors of urinary bladder: (A) Papilloma-papillary carcinoma; (B) Invasive papillary carcinoma; (C) Flat noninvasive carcinoma (CIS); (D) Flat invasive carcinoma

Abbreviation: CIS, carcinoma in situ.



Figs 21.47A and B: (A) Photomicrograph; (B) Diagrammatic. Low-grade papillary urothelial carcinoma with orderly appearance of transitional cells

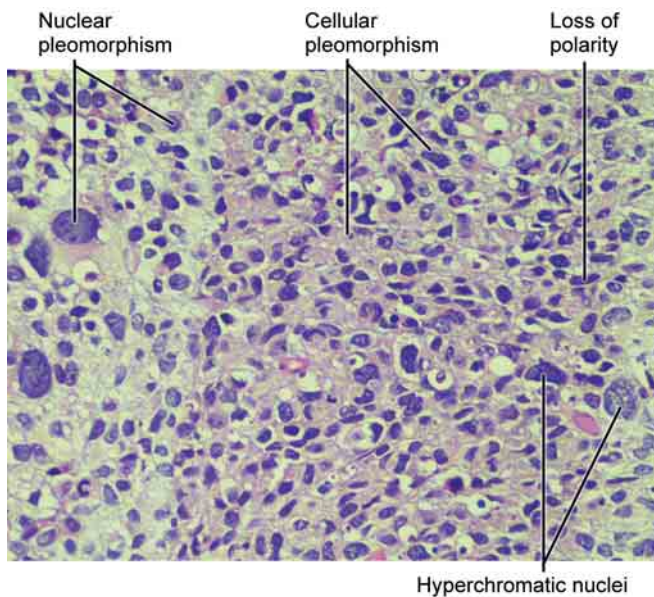


Fig. 21.48: High-grade urothelial carcinoma showing nuclear and cellular pleomorphism, hyperchromatic nuclei and loss of polarity

Exfoliated markers for detection of bladder cancer:

1. Newer markers: BTA test, urinary nuclear matrix protein (NMP22)- detects cancer specific proteins in urine (BTA/NMP22)
2. Hyaluronidase, Lewis-X antigen on exfoliated urothelial cells
3. Determination of telomerase activity in exfoliated cells.

TCC: Painless hematuria is the most common symptom.

TCC: Multifocal tumor and may recur.

- **Recurrences:** Urothelial tumors, irrespective of their grade may recur, usually at different sites than the original tumor.
- **Prognosis:** It depends on the histologic grade and the stage at the time of diagnosis.
- **Laboratory diagnosis:** Cytologic examinations of urine for malignant cells and biopsy of the tumor.

Male Genital Tract Disorders

PENIS

CARCINOMA IN SITU (CIS)

Q. Write short note on premalignant lesions of penis.

Two lesions in the external male genitalia show histological features of CIS namely: (1) Bowen disease and (2) Bowenoid papulosis. They are strongly associated with HPV infection (most commonly type 16).

Risk factors for invasive squamous cell carcinoma of penis: Bowen disease and rarely bowenoid papulosis.

Bowen Disease

- **Age and gender:** It occurs in the **genital region of both male and female**, usually **over the age of 35 years**. In male, it involves the **skin of the shaft of the penis and the scrotum**.

MORPHOLOGY

- **Gross:** It appears as a **single, thickened, gray-white and opaque plaque**. On the **glans and prepuce**, it may also appear as **single or multiple shiny red plaques**.
- **Microscopy**
 - **Epidermis** shows **proliferation with numerous mitoses** (few may be atypical).
 - The cells are **dysplastic** containing **large hyperchromatic nuclei** and lack of orderly maturation (**loss of polarity**).
 - However, the **basement membrane is intact** and there is sharp demarcation of the dermal-epidermal border.
- **Progression:** It may progress to **infiltrating squamous cell carcinoma** in about 10% of patients. Bowen disease may also be **associated with visceral cancer** (e.g. carcinoma of colon or breast).

Bowen disease: It may:

1. Progress to SCC
2. Associated with visceral cancer (e.g. carcinoma colon or breast).

Erythroplasia of Queyrat:

- Lesion of Bowen disease (i.e. carcinoma in situ) on the glans penis.
- Clinically and histologically similar to Bowen disease.

Bowenoid Papulosis

- **It occurs in sexually active adults.** In contrast to Bowen disease, it occurs at a **younger age** and the **lesions are multiple** (rather than solitary) **reddish brown papular lesions**.
- **Sites involved:** In men—glans and shaft of penis and in women—perineal and vulvar areas.
- **Microscopy:** Bowenoid papulosis is **indistinguishable from Bowen disease** and is also related to HPV type 16.
- **Fate of the lesion:** (1) Tendency toward spontaneous resolution, (2) spontaneous regression, and (3) very rarely invasive squamous cell carcinoma.

INVASIVE CARCINOMA

Penis: Squamous cell carcinoma and its precursor lesions may be associated with HPV infection.

Q. Write short note on carcinoma of penis.

- Squamous cell carcinoma of the penis is not a common malignancy.
- **Age:** Carcinomas are commonly found in between **40 and 70 years** of ages.

Carcinoma penis: Squamous cell carcinoma.

Etiology

Circumcision: Protective role against carcinoma of penis; decreases infection with HPV 16 and 18.

1. **Circumcision:** It has a **protective role**, and hence very rare among Jews and Moslems.
 - Circumcision is associated with **better genital hygiene**, and **reduces exposure to carcinogens** that may be concentrated in **smegma**.
 - It also **decreases** the likelihood of infection with potentially **oncogenic types of HPV** (HPV type 16 and HPV 18).
2. **HPV DNA** can be detected in squamous cancer of penis in about 50% of patients.
3. **Cigarette smoking** raises the risk of carcinoma of the penis.

Penis: Squamous cell carcinoma occurs on the glans or inner surface of the prepuce.

MORPHOLOGY

- **Site:** Squamous cell carcinoma of the penis usually develops on the **glans or inner surface of the prepuce** near the coronal sulcus.

Gross (Fig. 22.1)

Squamous cell carcinoma of penis

Two gross patterns: 1. Papillary, 2. Flat.

- Two macroscopic patterns of carcinoma are: Papillary and flat.
 1. **Papillary lesions:** They appear similar to condylomata acuminata and usually produce a **cauliflower-like fungating mass**.
 2. **Flat lesions:** They appear as **areas of epithelial thickening** accompanied by fissuring of the mucosal surface. **With progression**, it appears as an **ulcerated papule**.

Microscopy (Fig. 22.2)

- Both the papillary and the flat lesions show squamous cell carcinomas with varying degrees of differentiation. Microscopic features are similar to squamous cell carcinoma in other regions of the body (refer page 459).

Verrucous carcinoma: Exophytic well-differentiated variant of squamous cell carcinoma which has low malignant potential.

Verrucous carcinoma: Locally invasive, but they rarely metastasize.

Clinical Features

- Invasive squamous cell carcinoma of the penis present as a slowly growing and locally invasive lesion.
- They usually do not produce pain until they undergo ulceration and infection.
- They may metastasize to inguinal lymph nodes. The prognosis depends on the stage of the tumor.

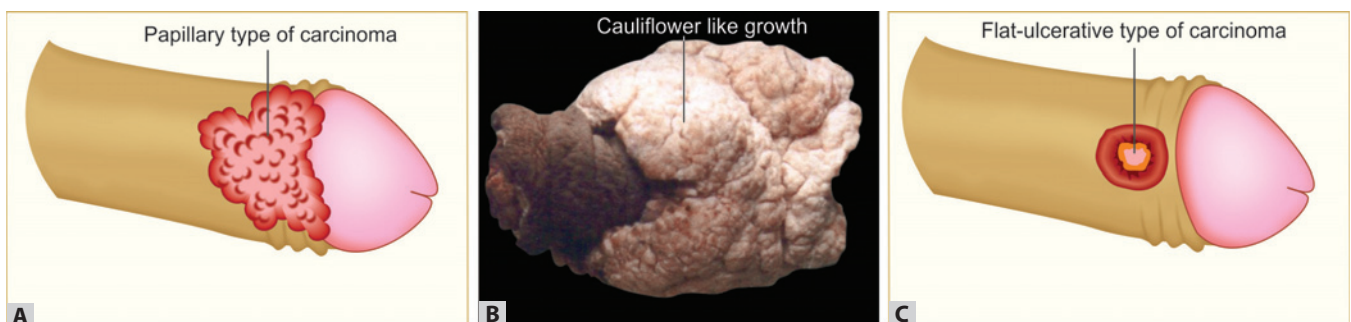
Carcinoma of penis: Most common lymph node involved is inguinal.

PROSTATE

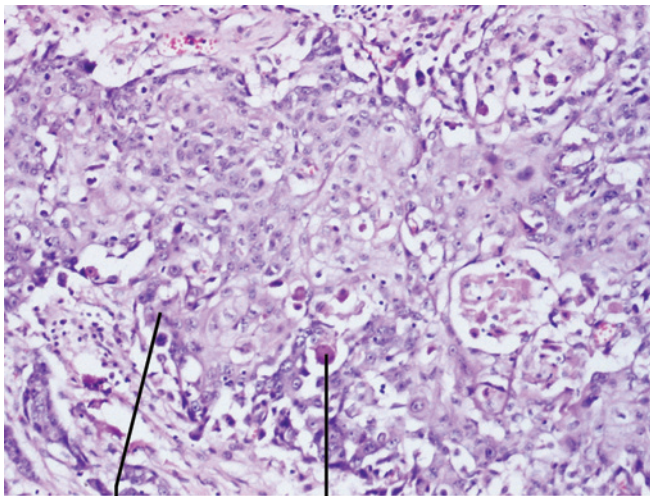
BENIGN PROSTATIC HYPERPLASIA OR NODULAR HYPERPLASIA

Q. Describe the pathogenesis of nodular hyperplasia/benign hyperplasia of prostate.

- Benign prostatic hyperplasia (BPH) is characterized by **hyperplasia of both prostatic stromal and epithelial cells**, which forms of large, fairly discrete (separate) nodules in the **periurethral region of the prostate**.



Figs 22.1A to C: Carcinoma of the penis. (A) (diagrammatic); (B) Papillary type of carcinoma (specimen). Shows glans penis deformed by a cauliflower-like growth; (C) Flat-ulcerative type of carcinoma (diagrammatic)



Malignant squamous cells infiltrating the stroma
Keratin formed by the tumor cells

Fig. 22.2: Microscopic appearance of squamous cell carcinoma of penis

- When the nodules become sufficiently large, they compress and narrow the urethral canal and cause **partial or complete obstruction of the urethra and urinary outflow**.
- **Age:** Very common disorder in men **50 years of age**.

BPH: Hyperplasia of both prostatic stromal and glandular epithelial cells.

BPH: Incidence is age related.

BPH: Most common cause of prostate enlargement in male above 50 years of age.

Etiology and Pathogenesis (Fig. 22.3)

In BPH, there are **increased number of epithelial cells and stromal cells** in the **periurethral area** of the prostate.

- **Epithelial cells:** **Increased number** of epithelial cells is not due to increased epithelial cell proliferation but mainly **due to reduction of the rate of cell death** → results in the **accumulation of senescent epithelial cells**.
- **Stromal cells:** **Increased** in due to **proliferation**.

BPH mostly originates from transitional zone prostate whereas carcinoma mostly arises from peripheral zone.

Role of Androgen

DHT: An androgen derived from testosterone plays an important role in the development of BPH.

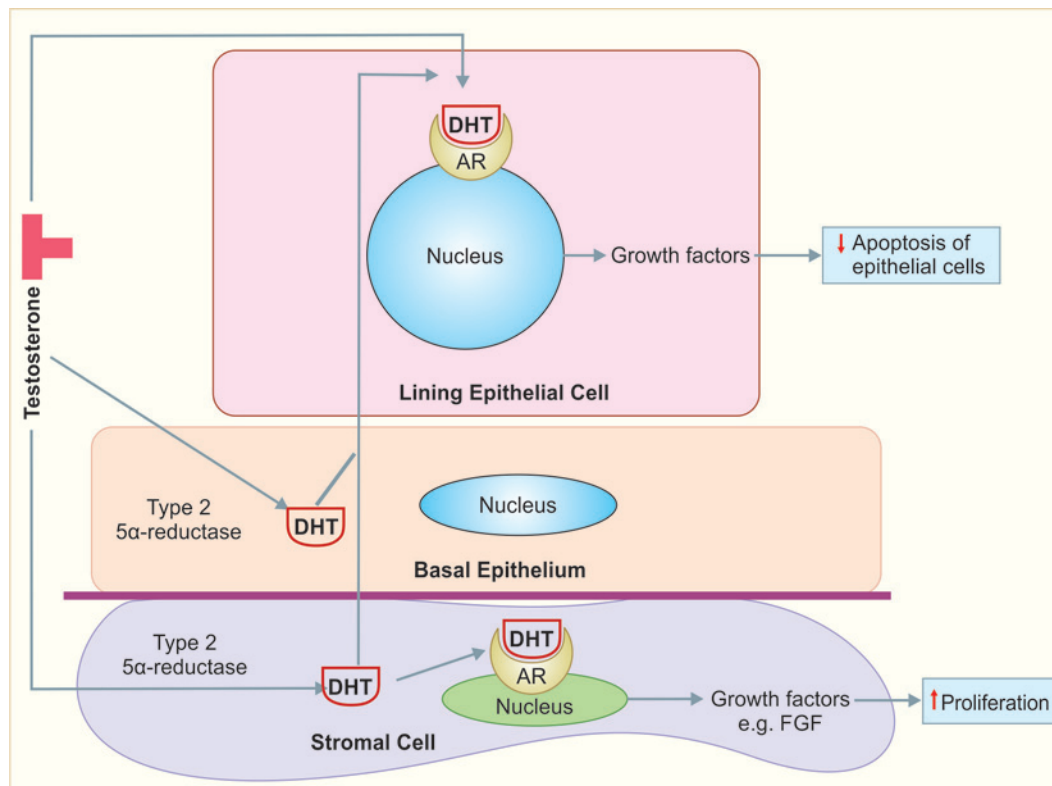


Fig. 22.3: Pathogenesis of prostatic hyperplasia. The stromal cells play a central role in the synthesis of dihydrotestosterone (DHT) from testosterone by type 2 5α-reductase

Abbreviations: AR, androgen receptor; FGF, fibroblast growth factor.

- Androgens are required for the development of BPH.
- It increases cellular proliferation and also inhibits cell death.
- **Source:** Main source of androgen (90% of total prostatic androgens) in the prostate is **dihydrotestosterone (DHT)**.
- **Synthesis:** DHT is formed in the prostate from the conversion of testosterone by the **type 2 5 α -reductase enzyme** present in the stromal cells and few basal cells. This enzyme is not present in the epithelial cells of the prostate. Thus, stromal cells are responsible for androgen-dependent growth of the prostate.
- **Actions of DHT:** DHT binds to the nuclear androgen receptor (AR) present in both epithelial and stromal cells of the prostate. DHT is more potent than testosterone because: (1) it has a higher affinity for AR and (2) forms a more stable complex with AR.
 - DHT binding to AR activates the transcription of androgen-dependent genes, which results in the increased production of many growth factors and their receptors. Most important growth factors are:
 - ♦ Fibroblast growth factor (FGF) family: FGF-7 (keratinocyte growth factor) is an important growth factor produced by stromal cells and mediate prostatic growth by paracrine mechanism.
 - ♦ Other growth factors: FGFs 1 and 2, and TGF- β cause fibroblast proliferation.

BPH may due to DHT-induced growth factors, which increases the proliferation of stromal cells and decreases the apoptotic death of epithelial cells.

MORPHOLOGY

Q. Describe the morphology of nodular hyperplasia of prostate.

Gross (Fig. 22.4)

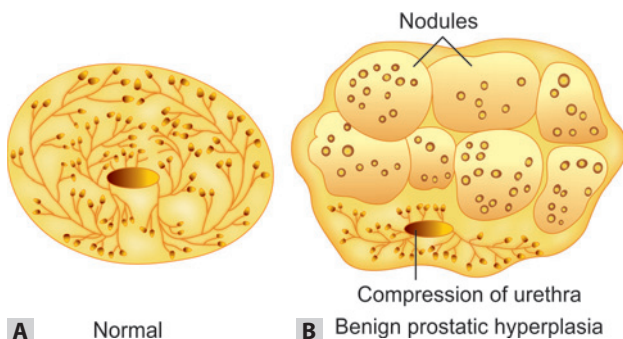
BPH: Involves periurethral/transitional zone of prostate.

- **Weight:** Usually weighs 2 to 4 times the normal weight and ranges from 60–100 g.
- **Sites of BPH:** Nodular hyperplasia begins in the **submucosa of the proximal urethra (transition zone)**. It usually involves the **both lateral lobes of prostate**.
- **Consequences:**
 - As the **nodules enlarge**, they may **compress** the centrally located **urethra** to a slit-like orifice and also the more peripherally located normal prostate.
 - Sometimes, **nodule may project up into the floor of the urethra** as a hemispheric mass directly beneath the mucosa of the urethra. This is called by the clinicians as **median lobe hypertrophy** (refer 1.5 and 22.4). It does not correspond to the anatomical middle lobe.
- **Cut-section:** It shows **multiple circumscribed nodules** without any true capsules and compresses the surrounding prostatic tissue, which creates a plane of cleavage between nodule and the normal prostatic tissue. **Nodules vary in color and consistency** depending on the predominant component.
 - Nodule with **predominant glands:** They appear **yellow-pink, honeycombed** and have **soft consistency**. Milky-white prostatic fluid oozes out from these areas.
 - Nodule with **predominant fibromuscular stroma:** They appear **pale gray, and are firm/tough in consistency**. These do not exude fluid.

Microscopy (Fig. 22.5)

BPH: Microscopically shows nodules composed of variable proportion of proliferating stroma and glands.

- **Nodularity:** It is the characteristic feature of BPH.
- **Composition of the nodules:** Proliferation of three types of cells in variable proportions.
 1. **Epithelial cells** (Acini and ductules): Their proliferation (adenomatous/glandular) leads to formation of small to large to cystically-dilated glands or acini.
 - ♦ **Glands are lined by two layers of cells**, an inner tall columnar and an outer basal layer of cuboidal or flattened epithelium.



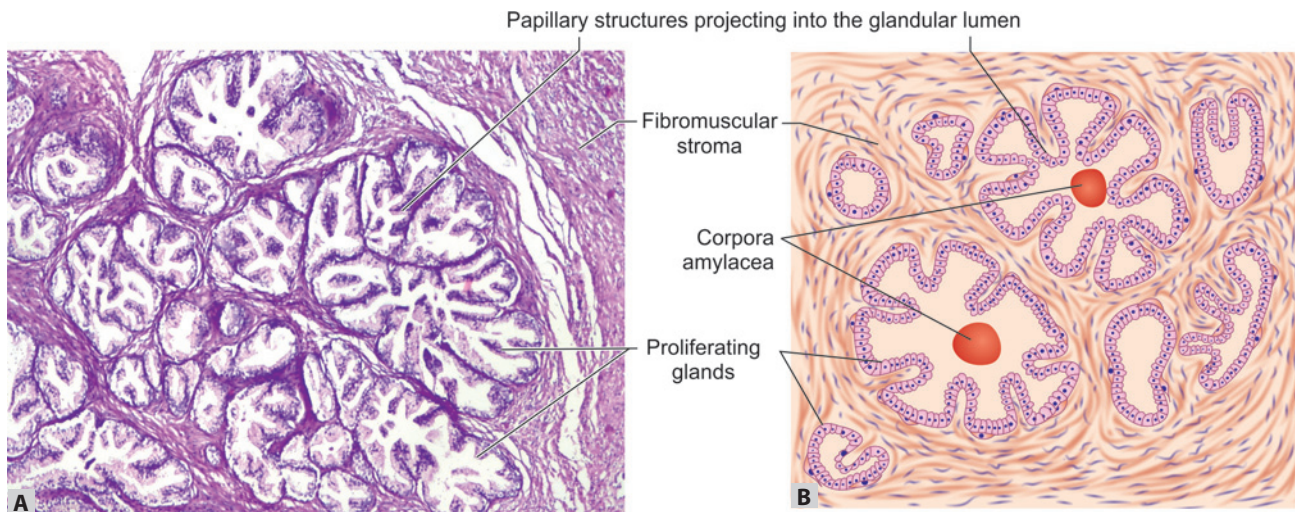
Nodular hyperplasia:

- Begins in the submucosa of proximal urethra
- Usually involves lateral lobes.

BPH: Composition of nodules:

1. Epithelial cells
2. Smooth muscle cells
3. Stromal fibroblasts.

Figs 22.4A and B: Schematic representation of cut-section of prostate. (A) Normal; (B) Benign prostatic hyperplasia consisting of well-defined nodules



Figs 22.5A and B: (A) Photomicrograph; (B) Diagrammatic appearance of benign prostatic hyperplasia showing proliferation of epithelial cells forming acini, few with papillary structures

- ♦ Characteristic feature is **papillary structures**, which project into the glandular lumen and are lined by columnar epithelial cells.
- ♦ **Corpora amylacea** (eosinophilic-laminated concretions) are commonly seen within the acini.

2. **Smooth muscle cells.**

3. **Stromal fibroblasts.**

- **Types of nodules:** Five types recognized and depend on the proportion of type of cells: (1) **stromal (fibrous)**, (2) **fibromuscular**, (3) **muscular**, (4) **fibroadenomatous**, and (5) **fibromyoadenomatous (most common)**.
- **Other findings:** (1) foci of lymphocyte infiltration, (2) areas of infarction, and (3) foci of squamous metaplasia at the edges of the infarcts.

BPH: Hyperplastic glands are lined by two cell layers: 1. Inner columnar 2. Outer flat basal cells.

Clinical Features

Nodular hyperplasia compresses the prostatic urethra and results in bladder outlet obstruction.

- Increasing urinary frequency
- Difficulty in urination
- Urine retention.

Complications (Fig. 22.6)

Q. Write short note on complications of nodular hyperplasia of prostate.

BPH: Not considered as a premalignant lesion.

- **Hypertrophy of bladder:** Obstruction to urinary outflow leads to hypertrophy and distension of the bladder accompanied by **retention of urine**. Trabeculation of bladder wall may develop, which can lead to **diverticula**.
- **Infection:** Inability to empty the bladder completely leads to residual urine in the bladder which is liable for infection (**cystitis**, and consequent ascending infection may cause **pyelonephritis**).
- **Consequences of obstruction:** Prolonged severe obstruction with back-pressure results in **hydronephrosis**, **hydroureter**, and ultimately death due to renal failure.

ADENOCARCINOMA OF PROSTATE

Carcinoma prostate: Most common cancer in males above 50 years.

Adenocarcinoma of the prostate is the **most common malignant tumor in males**.

Carcinoma of prostate:

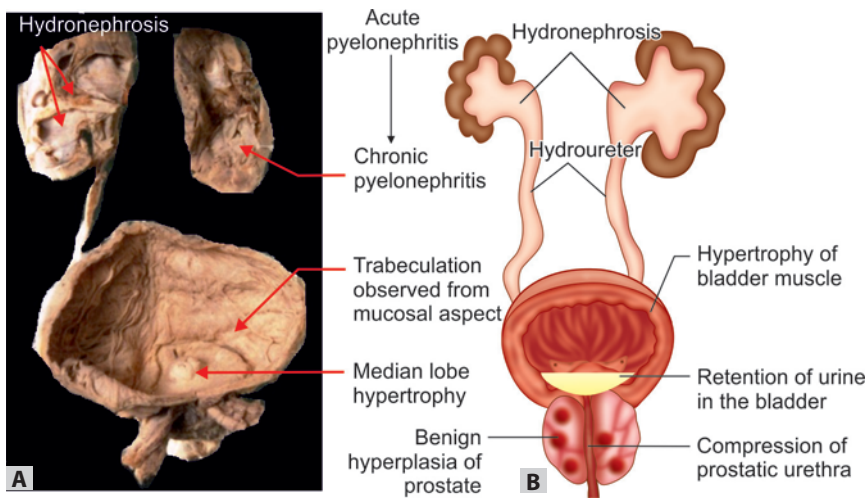
- Most common cancer in males
- Most common cause of bone secondaries in males.

Etiology and Pathogenesis

Carcinoma prostate: DTH dependent.

Factors Involved in Carcinoma of Prostate

- **Age:** It usually develops in men between **65 and 75 years of age**.
- **Environmental factors:** The increased incidence of carcinoma prostate upon migration from a low-incidence



Figs 22.6A and B: Complications of benign hyperplasia of prostate. (A) Gross appearance of hypertrophied bladder with a nodule projected up into the floor of the bladder. Both kidneys show hydronephrosis and chronic pyelonephritis; (B) Diagrammatic representation

Complications of BPH:

1. Hypertrophy of bladder
2. Cystitis
3. Pyelonephritis
4. Hydronephrosis
5. Bladder diverticula.

5 α -reductase inhibitors (e.g. finasteride) and α 1a receptor antagonists (e.g. tamsulosin) can be used for treatment of BPH.

region to one with a high-incidence point towards the role for environmental factors.

- **Dietary factors:**
 - Increased consumption of fats increases the risk.
 - Diets which prevent or delay prostate cancer: Lycopenes (found in tomatoes), selenium, soya products, and vitamin D.
- **Androgens:** Prostatic cancer growth depends on androgens.
 - Androgens bind to the androgen receptor (AR) and express of pro-growth and pro-survival genes.
 - Castration or treatment with anti-androgens induces disease regression.
- **Race:** Differences in prostate cancer risk among races were observed.
- **Family history:** Men with strong family history of prostate cancer have **two-fold risk** prostate cancer and develop cancer at an earlier age.
- **Hereditary factors:** Germline mutations of the tumor suppressor gene *BRCA2* and germline mutation in *HOXB13* (a homeobox gene encoding a transcription factor that regulates prostatic development) is associated with **increased risk** of prostate cancer.

Somatic Mutation in Prostate Cancer

Carcinoma prostate: *TPRSS2-ETS* fusion gene.

Prostate carcinoma develops as a product of combination of acquired somatic mutations and epigenetic changes.

- ***TPRSS2-ETS* fusion gene:** They are observed in 40 to 50% of prostate cancer.

- **Over-expression of ETS transcription factors:** It results in upregulation of matrix metalloproteases and makes normal prostate epithelial cells more invasive.
- **Epigenetic changes:** Hypermethylation of glutathione S-transferase (*GSTP1*) gene can predispose to cancer of prostate. *GSTP1* gene (located on chromosome 11q13) prevents damage from a wide variety of carcinogens.
- **Activation of oncogenes:** They activate PI3K/AKT pathway of cell proliferation.
- **Mutation that inactivate tumor suppressor gene *PTEN*:** *PTEN* acts as a brake on cell proliferation by PI3K.

GSTP1 gene: Located on chromosome 11q13 prevents damage from a wide variety of carcinogens.

MORPHOLOGY (FIG. 22.7)

Gross

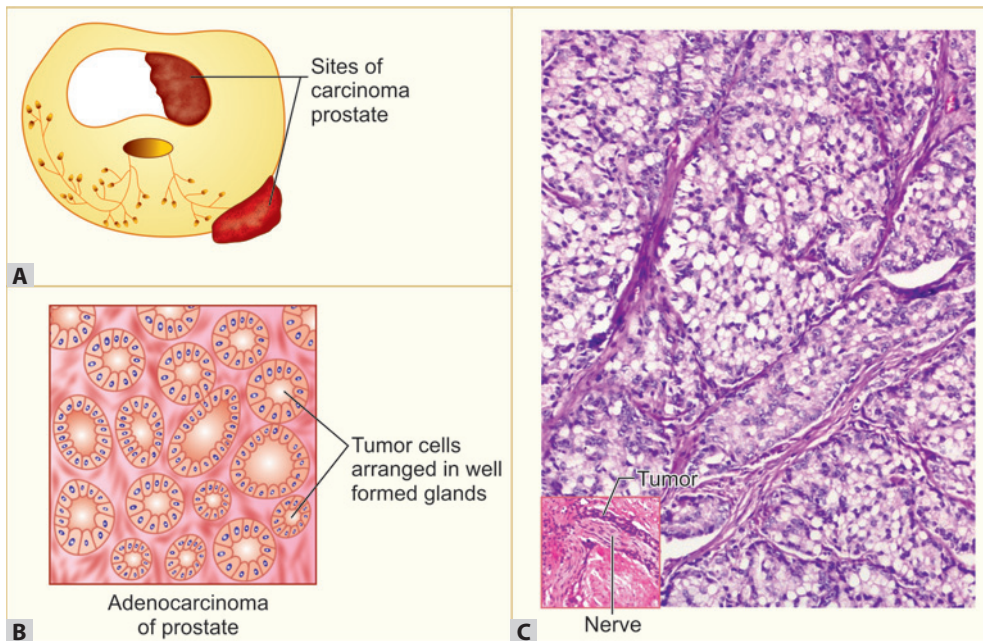
- **Site:** Carcinoma of the prostate arises in the **peripheral zone of the gland (70%)**, usually in a **posterior location**, and makes it palpable on rectal examination.
- **Cut-section:** Tumor tissue is **gritty and firm**. When tumor is embedded within the prostate, it may be more readily appreciated on palpation than by visualization.

Carcinoma prostate: Most commonly in the outer peripheral zone.

Carcinoma prostate: Peripheral in location and may be palpable by rectal examination.

Microscopy

Most prostate cancers are **adenocarcinomas** and consist of well-defined glandular patterns.



Figs 22.7A to C: Carcinoma of prostate. (A) Schematic gross; (B) Diagrammatic microscopy; (C) Microphotograph of low-grade prostate cancer consisting of back-to-back uniform-sized malignant glands. Inset shows perineural invasion

Carcinoma of prostate:

Hematogenous spread occurs:

- Mostly to bone (axial skeleton is the most common site with lumbar spine being most frequently involved)—osteoblastic in nature
- Visceral metastasis most commonly to lung>liver>adrenal glands.

Most common primary site of bone secondaries:

- In males: Carcinoma prostate
- In females: Carcinoma of breast.

Feature that differentiate benign and malignant prostate gland:

- Benign glands have two layers: Basal cells and columnar cells
- Cancer: Single layer and absence of basal cells.

• Glands:

- Neoplastic glands are usually smaller than benign glands—microacinar.
- In contrast to benign glands, prostate cancer glands are more crowded, and lack branching and papillary infolding.
- Glands are lined by a single uniform layer of cuboidal or low columnar cells.
- The outer basal cell layer, which is seen in benign glands is absent in cancer. This is used as criteria to distinguish benign and malignant prostate glands.

• Tumor cells:

- Uniform cuboidal or low columnar type.
- Cytoplasm ranges from pale-clear (similar to benign glands) to a distinctive amphophilic appearance.
- Nuclei are large and may contain one or more large nucleoli.
- Mild variation in nuclear size and shape may be seen, but pleomorphism is not marked.
- Mitotic figures are uncommon.
- **Immunohistological markers:** Differentiation of benign from malignant prostate glands is that benign glands have basal cells, whereas they are absent in glands in cancer. This can be detected by using various immunohistological markers to label basal cells. One of the immunohistochemical markers namely, α -methylacyl-coenzyme A-racemase (AMACR) is up-regulated in prostate cancer. Most of the prostate cancers are positive for AMACR.

Carcinoma prostate: Adenocarcinoma consists of neoplastic glands lined by single layer of tumor cells. Most are acinar type.

Carcinoma of prostate:

1. Neoplastic glands
 - Smaller
 - More crowded
 - Lack branching and papillary folding.
2. Diagnosis is based on the absence of outer basal cell layer.

Prostatic Intraepithelial Neoplasia (PIN)

- PIN consists of benign prostatic acini lined by cytologically atypical cells with prominent nucleoli.
- PIN is a precursor of invasive cancer.

Spread

Q. Write short note on spread of carcinoma of prostate.

1. Local spread:

- Invasion of prostatic capsule.
- **Perineural tumor invasion** (Fig. 22.7 C inset) both in the prostate and adjacent tissues.
- Spreads into periprostatic tissue, seminal vesicles, and the base of the urinary bladder.

2. Lymphatic spread:

- First to the obturator nodes.
- Later to iliac and to the para-aortic lymph nodes.
- Metastases to the lung are due lymphatic spread through the thoracic duct and through the prostatic venous plexus to the inferior vena cava.

3. Hematogenous spread:

- Mainly to the **bones of the axial skeleton**.
- The bony metastases are **osteoblastic** and strongly point towards prostatic cancer.
- The **bones involved, in descending order of frequency, are lumbar spine, proximal femur, pelvis, thoracic spine, and ribs**.
- Massive visceral dissemination is rare.

Carcinoma prostate: Osteoblastic metastases to axial skeleton (e.g. lumbar spine, pelvis).

Most other carcinoma bone metastases is osteolytic.

Grading and Staging

Carcinoma prostate: Gleason grading system is used which correlates stage and prognosis.

Grading of carcinoma prostate: Gleason system.

- **Grading and staging** are the **best prognostic predictors** of carcinoma of prostate.

Gleason Grading

- This is the most **widely used microscopic grading system** for adenocarcinoma of prostate.
- It is **based on the degree of glandular architectural differentiation** and the **growth pattern** of the tumor in relation to the stroma as identified at relatively low magnification.

Architectural patterns: It can be divided into two patterns:

1. **Primary pattern:** It is the **predominant tumor pattern**.
2. **Secondary pattern:** It is the **second most prevalent pattern** (if present).

Grading

- Gleason grade: Ranges from 1 to 5 with 5 having the worst prognosis
- Gleason score: Ranges from 2 to 10.
- Both the primary and secondary architectural patterns are **graded from 1 to 5**, with 1 being the most differentiated and 5 being undifferentiated.
 - **Grade 1 is the well-differentiated** tumor and consists of uniform and round neoplastic glands which form well-circumscribed nodules.

- By contrast, **grade 5** tumors consist of cords, sheets, and nests of tumor cells infiltrating the stroma **without glandular differentiation**.
- The other grades fall in between these two.
- If a tumor has only one histological pattern, then, both the primary and secondary patterns are given the same grade.

Gleason Score or Sum

Gleason score is used for grading of prostate cancer.

- The **combined Gleason grades** is called as Gleason score or sum. It is obtained by adding the two numbers of primary and secondary patterns.
- It ranges from 2 ($1 + 1 = 2$), which represents tumors uniformly composed of Gleason pattern 1 tumor, to 10 ($5 + 5 = 10$), which represents totally undifferentiated tumors.

Minimum Gleason score is 2 ($1 + 1$) and is most differentiated whereas maximum score is 10 ($5 + 5$), least differentiated.

Staging of Prostatic Cancer

- **TNM system** is used for staging.
- Staging is not only important for **predicting prognosis**, but also important in the selection of the **appropriate form of therapy**.

Clinical Course

- **Early stages**, it is **asymptomatic**. They are usually discovered during rectal examination or elevated serum PSA level.
- **Advanced** prostatic cancer **may present** with **urinary symptoms**, like dysuria, frequency, or hematuria.
- **Vertebral metastases may present** as **back pain** and has fatal outcome. **Detection of osteoblastic metastases by skeletal surveys or by radionuclide bone scanning is virtually diagnostic** of prostatic cancer.
- Digital rectal examination, transrectal ultrasonography and other imaging modalities has both low sensitivity and specificity.

Transrectal needle biopsy is needed to confirm the diagnosis.

Tumor Markers

Raised PSA:

- Carcinoma prostate
- BPH
- Prostatitis
- Infarct of prostate
- Instrumentation of the prostate
- Ejaculation.

Prostate-specific Antigen (PSA)

- Most important tumor marker used for diagnosis and management.
- PSA is produced by prostatic epithelium and only minute amounts of PSA circulate in the serum (serum level of 4 ng/mL is cutoff point between normal and abnormal).
- Raised blood PSA levels occur with localized as well as advanced prostatic cancer. But, PSA value may be normal or less.
- PSA is organ-specific, but not cancer-specific.
- Serial measurements of PSA are useful for assessing the response to therapy. For example, a rising PSA after radical prostatectomy or radiotherapy indicates recurrent or dissemination.

PSA: Organ-specific, but not cancer-specific.

PSA: Great value in assessing the response to therapy and detect recurrence.

Refinements in the Estimation and Interpretation of PSA

- **PSA density:** It is the ratio between the serum PSA value and volume of prostate gland.
- **PSA velocity:** It is the rate of change in PSA value with time.
- **Age-specific reference ranges.**
- **Ratio of free and bound PSA in the serum.**

PSA velocity of 0.75 ng/mL/year best distinguishes between cancer and benign lesions of prostate.

Prostatic Acid Phosphatase (PAP)

- It is secreted by prostatic epithelium. Its activity is 1,000 fold greater in prostate than in any other tissue
- It is elevated when the prostatic cancer has spread beyond prostate or metastasized.
- Not prostate specific and is increased in renal, liver and bone malignancies.

Other tumor markers: PCA is a noncoding RNA that is overexpressed in 95% of prostate cancers. Quantification of urine PCA3A is also used as diagnostic additional biomarker in patients suspected to have prostate cancer because of elevated PSA, but where prostate biopsy does not reveal cancer. The combination of urinary PCA3 with screening of urine for TMPRSS2-ERG fusion DNA.

TESTIS

TESTICULAR TUMORS

Q. Classify testicular tumors.

Classification (Box 22.1)

Testicular tumors: Most common cause of painless enlargement of testis.

Testicular neoplasms are divided into two major categories:

1. **Germ cell tumors:** They arise from germ cells and constitute about 95% of testicular tumors. Most of them are aggressive and can rapidly disseminate, but with current therapy most can be cured. From a clinical standpoint, germ tumors of the testis are subdivided into two broad categories:

Testicular germ cell tumors:

1. Seminomatous
2. Non-seminomatous.

- **Seminomatous tumors:** Remain localized to the testis for a long time. They spread mainly to paraaortic nodes and distant spread is rare.
- **Non-seminomatous tumors (NSGCTs)** tumors tend to spread earlier, by both lymphatics and blood vessels.

2. **Sex cord-stromal tumors:** These tumors arise from stroma and are generally benign.

BOX 22.1: Classification of testicular tumors

- I. **Germ cell tumors**
 - A. **Seminomatous tumors**
 1. Seminoma
 2. Spermatocytic seminoma
 3. Anaplastic seminoma
 - B. **Non-seminomatous tumors**
 1. Embryonal carcinoma
 2. Yolk sac (endodermal sinus) tumor
 3. Choriocarcinoma
 - C. **Teratoma**
 - Mature
 - Immature
 - Teratoma with malignant transformation
 - D. **Mixed germ cell tumors**
- II. **Sex cord-stromal tumors**
 - A. Leydig cell tumor
 - B. Sertoli cell tumor

Contd..

Contd..

Most common tumor of testis:

- Seminoma
- Mixed germ cell tumor

Most common testicular tumor in infant and children up to 3 years: Yolk sac tumor

Most common testicular tumor in prepubertal children: Teratoma

Most common testicular tumor >60 years: Lymphoma

Most common bilateral tumor

- Primary is seminoma
- Secondary is lymphoma.

GERM CELL TUMORS

Etiology and Pathogenesis

The exact etiology of testicular tumors is not known.

1. **Environmental factors:** They may play role in testicular germ cell tumors. The incidence of testicular tumors shows a geographic variation.
2. **Cryptorchidism:** About 10% of testicular germ cell tumors are associated with **undescended testis and is the most important risk factor**. The high incidence of testicular germ cell tumors may be due to its exposure to high temperature in the abdomen or inguinal region compared to that in the scrotum.

Cryptorchid testis: Incomplete descent of testis into scrotal sac.

Cryptorchid testis: Risk factor for seminoma (more for abdominal than for inguinal testis).

Cytogenetic abnormality in testicular germ cell tumor: Isochromosome p12.

Risk of testicular malignancy in undescended testes is **NOT REDUCED** by orchiopexy.

3. **Testicular dysgenesis syndrome (TDS):** It constitutes a spectrum of disorders and is one of the known risk factor for testicular germ cell tumors. This syndrome **includes cryptorchidism, hypospadias, and poor sperm quality**. These conditions might be related to in utero exposures to pesticides and estrogens.
4. **Genetic/family predisposition:** There is a strong family or genetic predisposition with the testicular germ cell tumors. The **cytogenetic abnormality observed is an additional fragment of chromosome 12 (isochromosome p12)**.
5. **Klinefelter syndrome:** It is associated with **50 times greater risk (than normal) for the mediastinal germ cell tumors**, but they **do not develop testicular tumors**.

6. **Time of malignant transformation:** It may occur **during fetal development** or in the **peripubertal period**.

Classification

Germ cell tumors of testis are subclassified into two groups.

1. **Seminomatous tumors:** Seminomas are the most common testicular tumor and constitute about 50% of all testicular germ cell neoplasms.
2. **Non-seminomatous tumors:** They are composed of undifferentiated cells.

Seminomas are the most common type of germ cell tumors (50%).

Germ cell tumors may contain: (1) only **single tissue component** or (2) **mixtures of seminomatous and non-seminomatous components**.

Germ cell tumors may progress through one of the two pathways:

- **Proceeded by intratubular germ cell neoplasia (ITGCN).**
- **Directly from germ cells without an in situ phase.**

Intratubular Germ Cell Neoplasia (ITGCN)

- ITGCN is **preinvasive form** of most invasive germ cell tumors.
- ITGCN **involves testes in a patchy manner**.
- **Genetic alterations:**
 - Gain of additional fragment short arm of chromosome 12 (**isochromosome p12**).
 - Activating mutations in the gene encoding the KIT receptor tyrosine kinase.
- **Progress:** All patients with ITGCN **subsequently develop invasive tumors**.

Genetic alterations in ITGCN:

- Isochromosome p12
- Mutations of *KIT* (oncogene).

MICROSCOPY

- **Seminiferous tubules** show **thick basement membranes, decreased diameter and without any sperms**.
- **Normal germ cells are replaced by neoplastic atypical primordial germ cells**.
- **Tumor cells:**
 - **Size:** Tumor cells are **larger than normal spermatogonia** and are about twice the size of normal germ cells
 - **Nuclei:** **Large and centrally placed, finely dispersed chromatin and prominent nucleoli**.
 - **Cytoplasm:** **Abundant, distinct cell membrane and clear cytoplasm**, which contains large amounts of glycogen.
 - **Immunohistochemistry:** **Placental alkaline phosphatase (PLAP) positive** on the plasma membrane.

ITGCN: PLAP positive.

SEMINOMA

Q. Write short note on seminoma.

Classification

1. Classical or typical
2. Anaplastic
3. Spermatocytic seminoma

Classical Seminoma

Seminomas: Most common type of germ cell tumor of testis.

- Seminomas are the **most common type of germ cell tumor** (~ 50% of germ cell tumors).
- **Age group:** Peak incidence is during **third decade (between 30 and 40 years)** and almost never found in infants or prepubertal children.
- **Female counterpart** occurs in the ovary is known as **dysgerminoma**.
- **Genetic alterations:**
 - Seminomas contain an **isochromosome 12p** (gain of additional fragment short arm of chromosome 12), and express *OCT3/4* and *NANOG*.
 - **Mutations of *KIT*** (oncogene) in about 25% of tumors. ***KIT* amplification** and overexpression may also occur without genetic defects.

Genetic alterations in seminoma:

- Isochromosome p12
- Mutations of *KIT* (oncogene)
- *KIT* amplification.

- **Origin:** Classic seminoma arises from **undifferentiated germ cells**.

MORPHOLOGY

Q. Write short note on morphology of seminoma.

Unless otherwise specified, the term seminoma refers to **classical or typical seminoma**.

Gross (Fig. 22.8)

Seminoma-gross: Bulky, solid, rubbery-firm, and bosselated tumors; cut section homogeneous, gray-white and lobulated.

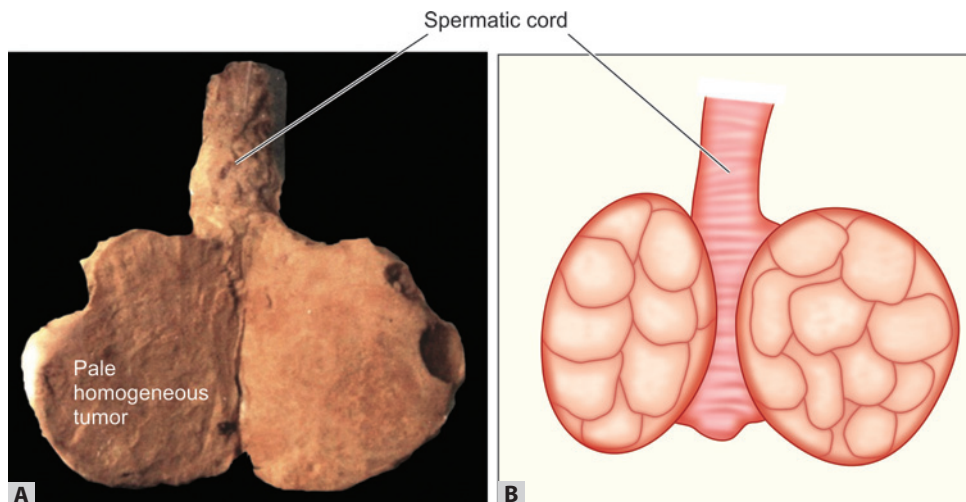
Seminomas are **bulky, solid, rubbery-firm, and bosselated tumors**.

- **Size:** It varies from **2 to 6 cm** and **testis is enlarged**, sometimes up to ten times of the normal testis.
- **Cut surface:**
 - Tumor is **homogeneous, gray-white** or grayish yellow, and **lobulated**.
 - Tumor is **sharply demarcated from normal testis**, which may be compressed, and atrophic.
 - Usually, the **tunica albuginea is not penetrated**.
 - **Areas of necrosis or hemorrhage are usually not seen**.

Microscopy (Fig. 22.9)

Seminoma-microscopy:

- Poorly demarcated lobules of uniform seminoma cells
- Delicate fibrous septa
- Septa infiltrated with lymphocytes and plasma cells.



Figs 22.8A and B: Gross appearance of seminoma of the testis. (A) Testis shows circumscribed, pale, fleshy, homogeneous mass; (B) (Diagrammatic) shows a lobulated tumor

Seminoma: Positive for:

1. KIT
2. PLAP.

AFP is **NEVER** elevated in seminoma.

Seminomas almost never occur in infants.

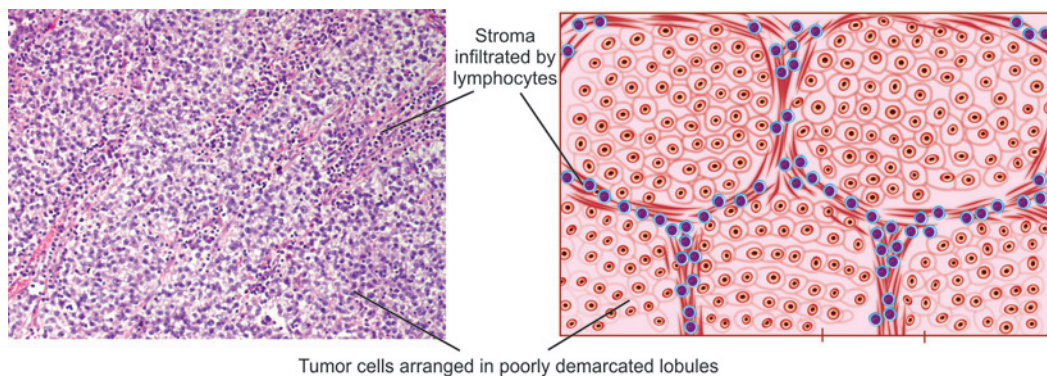


Fig. 22.9: Microscopy of seminoma shows seminoma cells divided into lobules separated by delicate septa with lymphocytic infiltrate

Seminoma appears microscopically similar to dysgerminoma of ovary.

1. **Pattern:** Tumor is composed of **sheets or nests or cords of uniform population of seminoma cells.**
2. **Classic seminoma (tumor) cells:** They have following features:
 - **Uniform single population** of cells that **resemble spermatogonia.**
 - Cells are **large, round to polyhedral** and have a **distinct cell membrane.**
 - **Cytoplasm:** May appear **pale and eosinophilic or clear (watery).** It contains varying amounts of **glycogen** and some **lipid.**
 - **Nucleus:** **Large, central vesicular nucleus** with **one or two prominent nucleoli.**
 - Mitoses vary in number.
3. **Stroma:**
 - Seminoma cells divided into **poorly demarcated lobules** by **delicate fibrous septa.**
 - Fibrous **septa** are **infiltrated with** moderate amount of **lymphocytes** and **plasma cells.**
4. **Rare features:**
 - **Syncytiotrophoblasts** (~15%) → **elevated hCG.**
 - Ill-defined granulomas with giant cells in the stroma.
5. **Immunohistochemistry:** Seminoma cells are **diffusely positive** for:
 - **KIT.**
 - **OCT4** and **placental alkaline phosphatase (PLAP)** on the plasma membrane.

Spread

1. **Local spread:**
 - Invasion of the testicular parenchyma.
 - Spread into rete testis.
 - Invasion of the epididymis.
2. **Lymphatic spread:** To abdominal lymph nodes.

Prognosis of Seminoma

Seminoma: Extremely radiosensitive and melts like ice.

- Extremely **radiosensitive.**
- **Remain localized** to the testis for long time (clinical stage I).
- **Metastases** mainly involve **lymph nodes.**
- **Hematogenous** spread occurs **late.**
- **Best prognosis.**

Anaplastic Seminoma

- Anaplastic seminoma is **cellular** and shows **nuclear pleomorphism** with more frequent **tumor giant cells** and many **mitotic figures.**
- **Not associated with worse prognosis** than classic seminoma.

Spermatocytic Seminoma

- Spermatocytic seminoma is a **distinctive tumor** of testis **both clinically and morphologically.**

Spermatocytic seminoma:

- Uncommon
- >65 years of age
- Slow growing
- Rarely metastasize
- Prognosis excellent.
- It is **rare** tumor (~1 to 2% of all testicular germ cell tumors).
- **Origin:**
 - Spermatocytic seminoma originates from **germ cells undergoing spermatogenesis.**
 - Represent **more differentiated** type of germ cell tumor.
- **Age:** Usually **above 65 years** of age.

MORPHOLOGY**Gross**

- Soft and pale gray.
- Cut surface may show mucoid cysts.

Microscopy

Spermatocytic seminoma: Three types of cells

1. Small lymphocyte-like
2. Intermediate
3. Giant.

Tumor cells: Three types:

- **Smaller lymphocyte-like cells:** These cells have a thin rim of eosinophilic cytoplasm. They resemble secondary spermatocytes.
- **Medium-sized intermediate cells:** They show eosinophilic cytoplasm and round nucleus and they are the **most numerous cells seen**.
- **Large cells:** Scattered giant cells, either uninucleate or multinucleate may be seen.

The chromatin in some tumor cells is filamentous in appearance, similar to that seen in the meiotic phase of non-neoplastic spermatocytes (**spireme chromatin**).

Spermatocytic seminoma:

Tumor cells have nucleus with spireme chromatin.

Prognosis

- **Slow-growing tumor:** It does not produce metastases.
- **Prognosis:** Excellent.

NONSEMINOMATOUS GERM CELL TUMORS**Embryonal Carcinoma**

- **More aggressive than seminomas.**
- **Age group:** 20 to 30 years.

MORPHOLOGY**Gross**

- **Size:** Small.
- **Cut surface:**
 - Has a **variegated appearance**.
 - **Poorly demarcated** at the margins.
 - **Areas of hemorrhage or necrosis** are common.
 - Tumor extends into tunica albuginea, epididymis or spermatic cord.

Microscopy

- **Pattern:**
 - The tumor cells are arranged in **alveolar or tubular or papillary** patterns. But they lack the well-formed glands with basally situated nuclei and apical cytoplasm.
 - More undifferentiated tumor show sheets of cells.
- **Tumor cells:**
 - Cells have an **epithelial appearance** and are **large and anaplastic**.
 - **Cell borders** are usually **indistinct**.
 - Considerable **variation in cell and nuclear size and shape**.
 - **Nuclei** are **hyperchromatic** with **prominent nucleoli**.
 - **Mitotic figures** and **tumor giant cells** are **frequent**.
- **Immunohistochemistry:** **Positive for cytokeratin and CD30, and negative for KIT.**

Embryonal carcinoma:

Positive for:

1. Cytokeratin
2. CD30

Negative for KIT.

Teratoma**Q. Write short note on teratoma of testis.**

Teratoma: Germ cell tumor arising from totipotent cells, which has capacity to differentiate into any of the germ cell layer (ectoderm, endoderm and mesoderm).

- Teratoma is a group of complex testicular tumors **composed of tissues derived from more than one germ layer** (ectoderm, mesoderm and endoderm), i.e. two or more than two germ layers.
- Pure teratomas constitute about 2 to 3% of germ cell tumors, but teratomas mixed with other germ cell tumors form about 45% of germ cell tumors.
- **Age:**
 - Can occur at **any age from infancy to adult life**.
 - Pure teratomas are common in infants and children. Rare in adults.

MORPHOLOGY**Q. Write short note on morphology of teratoma of testis.****Classification**

Depending on the morphological features, teratomas can be categorized into:

1. **Mature**
2. **Immature**
3. **Teratoma with malignant transformation.**

Gross (Fig. 22.10)

All types of teratoma almost have similar gross appearance.

- **Size:** Usually large, and range from 5 to 10 cm in diameter.
- **Appearance:** It has a **heterogeneous appearance** with **solid**, sometimes cartilaginous, and **cystic areas**. This appearance is due to the various tissues derived from more than one germ cell layers.
- Presence of hemorrhage and necrosis usually point towards the mixed tumors like embryonal carcinoma, choriocarcinoma, or both.

Microscopy (Fig. 22.11)

Teratoma:

1. Mature
2. Immature
3. Teratoma with malignant transformation.

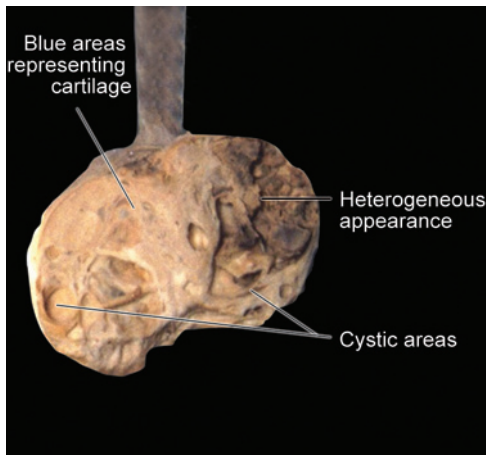
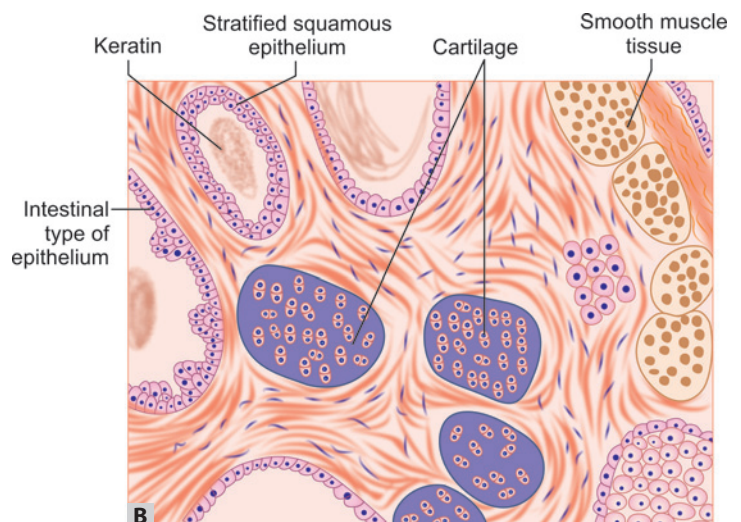
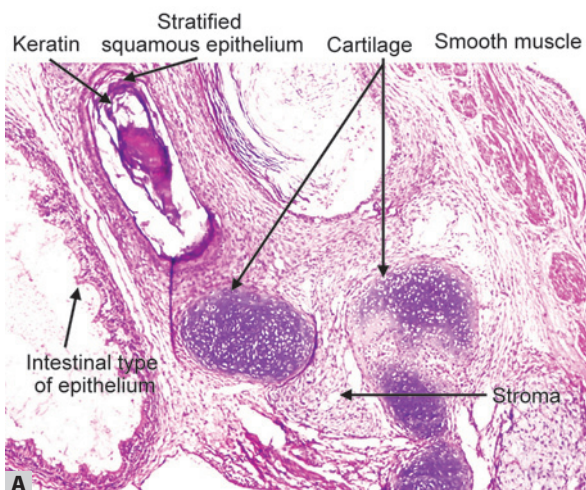


Fig. 22.10: Teratoma of testis. Cut section of testis has a variegated appearance with cysts, reflects the multiplicity of tissue found microscopically



Figs 22.11A and B: (A) Photomicrograph; (B) Diagrammatic appearance of teratoma of the testis consisting of a disorganized collection of glands, cartilage, smooth muscle, and stroma

1. Mature teratoma:

- Consists of a **heterogeneous, Helter-Skelter collection of differentiated cells or organoid structures** derived from **more than one germ cell layer**. These include:
 - **Ectoderm:** For example, skin (clusters of squamous epithelium, sweat and sebaceous glands, hair), tooth enamel, brain substance (neural tissue, glia).
 - **Mesoderm:** Example, smooth muscle bundles, islands of cartilage, bone, fat, blood vessels and lymphatics.
 - **Endoderm:** Example, respiratory tract (bronchial or bronchiolar epithelium), gut (bits of intestinal wall) structures reminiscent of thyroid gland.
- Above tissue elements are all embedded in a **fibrous or myxoid stroma**.
- **All the elements are mature**, which resemble various adult tissues.

2. Immature teratoma:

- Composed of tissues, which microscopically resemble **embryonal and immature fetal tissue**.
- **Immature tissues** mixed with some **mature tissues** derived from the **two or three germ layers**.
- Immature elements include **immature cartilage, neuroepithelium (neuroepithelial rosettes and immature glia), bone, muscle, and others**.

Immature teratoma: Consists of immature tissue elements mixed with mature elements derived from any of the germ cell layer (ectoderm, endoderm and mesoderm).

3. Teratoma with malignant transformation:

- It is the **development of malignant non-germ cell tumors in a teratoma**.
- They are rare.
- Malignancy transformation may occur in any tissue derived from one or more germ cell layers. For examples, **squamous cell carcinoma**, mucin-secreting adenocarcinoma and sarcoma.
- **Significance:** When the non-germ cell malignancy spreads outside of the testis, it does not respond to chemotherapy.

Q. Differences between seminoma and nonseminomatous germ cell tumors.**TABLE 22.1:** Differences between seminomatous and nonseminomatous tumors of testis

Characteristics	Seminomatous	Nonseminomatous
Characteristic of tumor	Remain localized to testis for long time	Some of them spread rapidly
Aggressiveness	Not very aggressive	Aggressive
Common mode of spread	By lymphatics	By hematogenous
Metastasis	Does not occur early	Early metastasis
Radiosensitivity	Highly radiosensitive	Radioresistant
Prognosis	Good	Poor

Dermoid cyst: A form of teratoma are common in the ovary, rare in the testis.

Prognosis of Teratoma

All postpubertal teratomas (both mature and immature): Considered malignant and are capable of metastasis.

- **Children:** Mature teratomas are usually benign.
- **Postpubertal:** All teratomas (both mature and immature) are considered malignant and are capable of metastasis. Thus, in a postpubertal male, it is not important to identify immature tissues in a testicular teratoma.

Differences between seminomatous and nonseminomatous tumors of testis are presented in Table 22.1.

Extragenital site of germ cell tumors:

1. Mediastinum (commonest)
2. Retroperitoneum
3. Pineal gland.

Mixed Tumors

- Mixed tumors are composed of mixtures of more than one of the “pure” patterns of germ cell tumors.
- Constitute ~ 60% of testicular tumors.
- **Common mixtures** are:
 - Teratoma, embryonal carcinoma, and yolk sac tumor.
 - Seminoma with embryonal carcinoma.
 - Embryonal carcinoma with teratoma (teratocarcinoma).
- **Prognosis:** Depends on the more aggressive component in the mixed tumor.

Clinical Features

Testicular tumor: Any solid testicular mass should be considered neoplastic unless otherwise proved.

- **Painless enlargement** of the testis.
- **Biopsy of a testicular neoplasm is contraindicated** because of risk of tumor spillage.
- Standard treatment of a solid testicular tumor is radical orchiectomy.

Spread of Testicular Tumors**Q. Write short note on spread of testicular tumors.**

Testicular tumor metastasis: Retroperitoneal and para-aortic nodes and not inguinal lymph nodes.

1. **Lymphatic spread:**
 - All types of **malignant testicular tumors spread through lymphatics.**
 - First, spreads to **retroperitoneal para-aortic nodes.**
 - Later, it may spread to mediastinal and supraclavicular nodes.
2. **Hematogenous spread**
 - To the **lungs, liver, brain, and bones**
 - Microscopic appearance of metastases may be different from that of the primary testicular tumor. For example, an embryonal carcinoma of testis may show a teratomatous element in the secondary deposits.

Biopsy of testicular neoplasm is associated with risk of tumor spillage, therefore, radical orchiectomy should be done on presumption of malignancy.

FNAC contraindicated in testicular tumors—it may result in inguinal lymph node metastasis.

Tumor Markers (Table 22.2)

- Germ cell tumors of the testis may **secrete hormones and enzymes**, which can be detected in blood.
- Tumor markers include **hCG, AFP, and lactate dehydrogenase.**

TABLE 22.2: Tumor markers of testicular germ cell tumors

Type of tumor	Tumor marker
Seminoma	hCG, placental alkaline phosphatase
Teratomas and teratocarcinoma	AFP, hCG
Embryonal carcinoma	CEA
Yolk sac tumor	AFP
Choriocarcinoma	β -hCG

Abbreviations: AFP, α fetoprotein; hCG, human chorionic gonadotropin; CEA, carcinoembryonic antigen.

- They are **valuable in the diagnosis and management** of testicular neoplasms.
 1. **Lactate dehydrogenase:** The level of raised lactate dehydrogenase **correlates with the mass of tumor cells**, and can be **used to assess tumor burden**.
 2. **AFP and hCG:** **Marked elevation** of these markers found in more than 80% of individuals **with NSGCT**.

Germ cell tumor markers:

1. LDH
2. hCG
3. AFP.

- ♦ AFP produced by yolk sac tumor.
- ♦ hCG levels: Raised in tumor with choriocarcinoma elements. Seminomas with syncytiotrophoblastic giant cells may also cause mild elevation of hCG levels.

• **Importance of tumor markers:**

- Evaluation of testicular masses.
- Staging of testicular germ cell tumors.
- Assessing tumor burden.
- Monitoring the response to therapy.

Pure choriocarcinoma: Most aggressive testicular tumor.

Yolk sac tumor of testis:

- Raised α -fetoprotein level (AFP).
- Schiller-Duval bodies.

Choriocarcinoma: Elevated hCG.

Female Genital Tract Disorders

CERVIX

WHO (2014) CLASSIFICATION OF TUMORS OF UTERINE CERVIX (BOX 23.1)

BOX 23.1: WHO (2014) Classification of tumors of uterine cervix (abridged)

Epithelial tumors

- Squamous cell tumors and precursors
 - Squamous intraepithelial lesions
 - ◆ Low-grade squamous intraepithelial lesions (LSIL)
 - ◆ High-grade squamous intraepithelial lesions (HSIL)
 - Squamous cell carcinoma
 - ◆ Keratinizing
 - ◆ Non-keratinizing
- Glandular tumors and precursors
 - Adenocarcinoma in situ
 - Adenocarcinoma
- Neuroendocrine tumors
 - Low-grade neuroendocrine tumor: Carcinoid tumor
 - High-grade neuroendocrine carcinoma:
 - ◆ Small cell neuroendocrine carcinoma (formerly termed small cell carcinoma)
 - ◆ Large cell neuroendocrine carcinoma

Mesenchymal tumors

- Benign: Leiomyoma
- Malignant: Leiomyosarcoma

Other tumors

CERVICAL INTRAEPITHELIAL NEOPLASIA (SQUAMOUS INTRAEPITHELIAL LESIONS)

Q. Define and write short note on cervical intraepithelial neoplasia (CIN).

CIN: Spectrum of intraepithelial changes ranging from minimal atypia to marked atypia without invasion of stroma.

Definition: Cervical intraepithelial neoplasia (CIN) is defined as a **spectrum of intraepithelial changes**, which **begins from minimal atypia** and **progresses through** more-marked stages of **intraepithelial abnormalities to invasive squamous cell carcinoma**.

- Age: CIN generally occurs **under the age of 40**.

Etiology and Pathogenesis of CIN and Carcinoma of Cervix

Risk factors: Common risk factors of CIN and carcinoma of cervix include: (1) human papilloma virus, and (2) environmental factors.

Risk factors for CIN and carcinoma cervix related to HPV exposure:

1. Early age at first intercourse
2. Multiple sexual partners
3. High parity
4. High-risk HPV (16 and 18).

Etiology and pathogenesis have common features for both CIN and carcinoma of cervix and are discussed together.

Human Papillomavirus Infection

Q. Discuss the role of HPV in carcinoma cervix.

Human papillomavirus infection (HPV) is a sexually transmitted DNA virus. Following features are associated with increased exposure to HPV and increased incidence of both CIN and cervical cancer. These features point that HPV is a major risk factor. Increased incidence of CIN and carcinoma cervix is observed in women with:

- **Multiple sexual partners**
- **First intercourse at young age**
- **High parity**
- **A male partner having multiple previous or current sexual partners**
- **Persistent infection** with a high oncogenic risk HPV, e.g. **HPV 16 or HPV 18**
- **Inefficient immune response:** Most HPV infections are transient, which are eliminated by the immune response and immunosuppression may result in persistent HPV infection.

Classification of HPV

HPV types: Low and high oncogenic risk.

CIN and carcinoma cervix: HPV is the major risk factor.

A. Low oncogenic risk:

Low oncogenic risk HPV: Virus replicates within the host cell leading to death of infected cell.

- Causes **condyloma acuminatum** in vulva, perineal and perianal region.
- **HPV genome** is maintained in a **nonintegrated free episomal** (extrachromosomal) form.
- **Virus freely replicates** and **causes the death of the host cell** and it is **known as productive infection**.

Condyloma is caused by HPV types: 6 and 11.

B. High oncogenic risk:

HPV high risk: Types 16 and 18.

- There are about 15 types of high oncogenic risk HPVs. Among these types, **HPV 16 and HPV 18 are the most important in cervical carcinogenesis**. Others include HPV 31 and 33.
- Can also cause **squamous cell carcinoma** of the **vagina, vulva, penis, anus, tonsil and oropharynx**.
- **Integration into the host DNA:** In cancers, the HPV genome (viral DNA) is integrated into the genome

of the host cell, which is important for malignant transformation. **Integration causes:**

- **Overexpression of the oncoproteins E6 and E7.**
- **Genomic instability** in the host cell.

HPV: Integration of viral DNA into the host DNA is necessary for malignant transformation of the host cell.

Features of infection by HPV

HPV infects:

1. Immature basal cells of squamous epithelium
2. Immature metaplastic squamous cells
3. Damaged surface epithelial cells of squamous epithelium.

Even though **HPV is a causative factor** for CIN and cancer of the cervix, **only few infected will develop cancer**. Most women infected with HPV clear the infection by immunological mechanisms.

- **Longer the duration of infection, higher the risk of CIN** and subsequent **carcinoma**. Infections with high oncogenic risk HPVs last longer than infections with low oncogenic risk HPVs.
- **HPVs infect immature basal cells of the squamous epithelium, or immature metaplastic squamous cells** present at the squamocolumnar junction.
- **Infects only damaged surface epithelium and not the mature intact superficial squamous cells**. In areas of epithelial breaks or damage, the HPV can reach the immature cells in the basal layer of the epithelium.
- **Replication occurs in the maturing nonproliferating squamous cells which normally are arrested in the G1 phase of the cell cycle** (though the virus infects only the immature squamous cells). However, these mature cells actively progress through the cell cycle when infected with HPV by using the host cell DNA synthesis machinery to replicate its own genome.
- **HPV has to induce DNA synthesis** and must **reactivate the mitotic cycle** in such nonproliferating cells. Viral replication results in a cytopathic effect, **“koilocytic atypia”**, consisting of **nuclear atypia** and a **cytoplasmic perinuclear halo**.

Oncogenesis by HPV

High-risk HPV: Express E6 and E7 oncoproteins → oncogenesis.

High-risk HPV types overexpress **E6 and E7 oncoproteins** that are responsible for the oncogenesis (refer page 203-204 and Fig 7.27).

- **Actions of E7 protein of HPV:** (1) Inactivation of RB and inhibition of cyclin-dependent kinase inhibitors (e.g. p21 and p27). These two actions increases progression of cell cycle and impair the ability of cells to repair DNA damage.

- **Actions of E6 protein of HPV:** (1) Degradation of the tumor suppressor protein p53 and up-regulates the expression of telomerase → leads to cellular immortalization.
- **Integration of viral DNA into the host cell genome:** In most cancers. The effects include (1) increased expression of E6 and E7 genes, (2) dysregulation of oncogenes near the sites of viral insertion (e.g. MYC).
- **Extrachromosomal (episomal) form of viral DNA:** It is observed in precursor lesions associated with high-risk HPVs and in condylomata associated with low-risk HPVs.

Environmental Factors

Risk factor for carcinoma of cervix:

- HPV
- Cigarette smoking
- Immunodeficiency.

Only HPV infection is not sufficient for carcinogenesis and it acts in association with the environmental factors. The environmental factors include:

- **Presence of co-carcinogens:** Cigarette smoke, which contains polycyclic hydrocarbons, acts as a co-carcinogen.
- **Coexisting microbial infections.**
- **Dietary deficiencies.**
- **Hormonal changes.**
- **Use of oral contraceptives.**

Consequences of HPV infection in the cervix are shown in Figure 23.1.

Tumors caused by HPV:

- CIN
- Carcinoma cervix
- Adenocarcinoma or adenosquamous carcinoma
- Neuroendocrine carcinoma.

Classification of Cervical Precancers (Fig. 23.2)

Presently used classification of cervical precancer is Bethesda system.

- Terminologies, namely CIN, dysplasia, CIS, and squamous intraepithelial lesion (SIL) are commonly used interchangeably. The classifications presently being used is **Bethesda system** squamous intraepithelial lesion.

Carcinoma In Situ (CIS) System

- It is the oldest classification in which **mild dysplasia** is at one end of the spectrum and severe **dysplasia/carcinoma in situ** on the other end.

Cervical Intraepithelial Neoplasia Classification

- According to this, mild dysplasia is termed **CIN I**, moderate dysplasia **CIN II**, and severe dysplasia termed as **CIN III**.

Squamous Intraepithelial Lesion

Bethesda system

- LSIL
- HSIL.

- The **Bethesda system** for Reporting Cervical/Vaginal Cytologic Diagnoses, groups these lesions into **low- and high-grade squamous intraepithelial lesions**.
 - **Low-grade squamous intraepithelial lesion (LSIL):** CIN I is renamed as low-grade squamous intraepithelial lesion (LSIL)
 - ◆ Associated with productive HPV infection.
 - ◆ Most LSILs regress spontaneously.

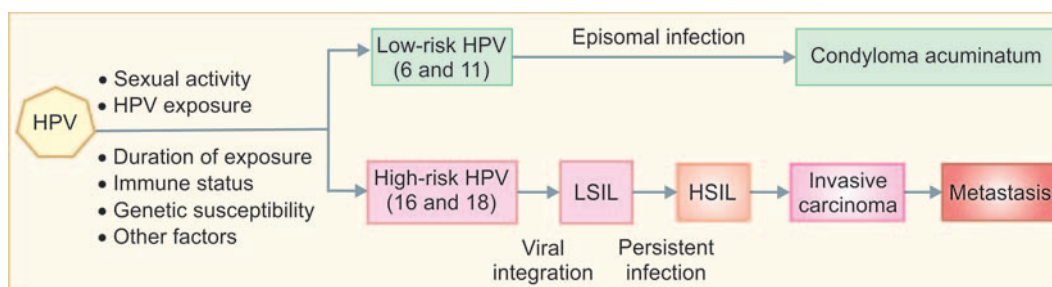


Fig. 23.1: Consequences of HPV infection in the cervix

Abbreviations: LSIL; low-grade squamous intraepithelial lesion, HSIL; high-grade squamous intraepithelial lesion

- **High-grade squamous intraepithelial lesion (HSIL):** CIN II and CIN III are combined into one category and are known as high-grade squamous intraepithelial lesion (HSIL).

♦ HSIL tend to progress.

Etiology and pathogenesis: Refer page 658-660.

MORPHOLOGY

Q. Write short note on morphology of carcinoma in situ/squamous intraepithelial lesion.

Squamous Intraepithelial Lesion (SIL)

Spectrum of morphologic changes range from **normal, low-grade to high-grade SIL** (Fig. 23.2).

Diagnosis of SIL: It is based on identification of atypical immature squamous cells that have following features:

1. **Nuclear atypia:** The characteristics of nuclear atypia are:
 - **Nuclear enlargement.**
 - **Pleomorphic nuclei:** Variation of nuclear sizes and shapes.
 - **Hyperchromasia:** Characterized by dark staining of nuclei.
 - **Coarse chromatin granules.**
2. **Nuclear cytoplasmic ratio:** Increased.
3. **Loss of polarity.**
4. **Cytoplasmic change:** The nuclear atypia mentioned above may be accompanied by perinuclear cytoplasmic halo, which are termed as **koilocytic atypia** (Figs 23.3A to C). The **koilocyte** (from the Greek *koilos*, hollow) is a **superficial or intermediate mature squamous cell** characterized by:
 - **Cytoplasm:** Sharply outlined **perinuclear halo (vacuolation)** formed **due to extensive cytoplasmic destruction** by a HPV-encoded protein E5 that is localized to the membranes of the endoplasmic reticulum. **Peripheral cytoplasm is dense and shows irregular staining.**
 - **Nucleus:** **Enlarged, wrinkled** with an **undulating** (raisin- or prune-like) **nuclear membrane** and **rope-like chromatin** pattern.

Koilocytes are absent in many cases of high-grade dysplasia and all invasive cancers.

Koilocyte: Superficial or intermediate mature squamous cell with perinuclear halo and enlarged, wrinkled nuclear membrane.

Raisin nucleus:

1. Koilocyte
2. Clear cell RCC.

Markers of actively dividing cells (e.g. Ki-67) are normally restricted to the actively dividing cells of basal layer of the epithelium. With HPV infection, E6 and E7 proteins of HPV prevent arrest of cell cycle arrest in the upper portion of the epithelium resulting in replication of these cells. Thus, superficial cells express markers, such as Ki-67. Disturbed growth regulation also causes overexpression of p16, a cyclin-dependent kinase inhibitor. Ki-67 and p16 staining are show high correlation with HPV infection and are useful for confirming the diagnosis in doubtful cases of SIL.

Koilocytotic atypia:

1. Nuclear atypia
2. Perinuclear vacuolization
3. Caused by HPV
4. Considered as viral “cytopathic” effect.

Koilocyte: Effect of HPV on squamous cells.

Grading of SIL

- Squamous intraepithelial lesion is graded as LSIL (low) and HSIL (high grade) based on expansion of the immature cell layer from its normal basal location.
- **LSIL:** If the **atypical, immature squamous cells** are **confined to the lower one-third of the epithelium**, the lesion is graded as LSIL.
- **HSIL** (Fig. 23.3): If the **atypical, immature squamous cells** expand to **involve lower two-thirds of the epithelium**, the lesion is graded as HSIL.

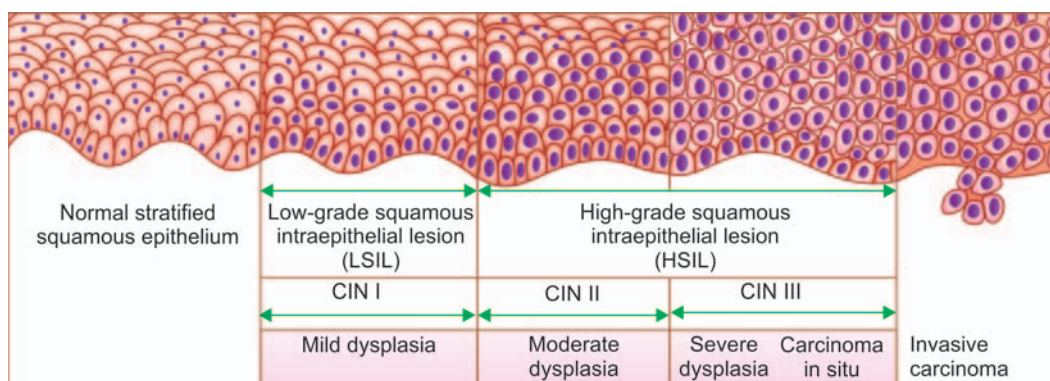
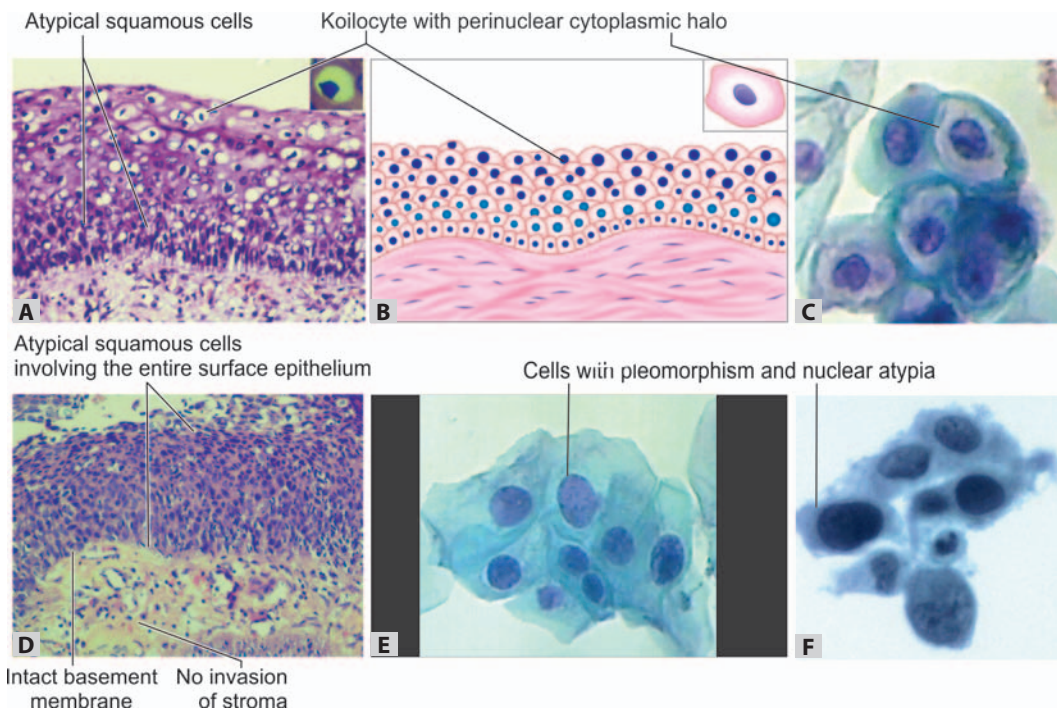


Fig. 23.2: Spectrum of cervical intraepithelial neoplasia with normal stratified squamous epithelium for comparison. LSIL (CIN I/mild dysplasia); HSIL (CIN II and CIN III/severe dysplasia and carcinoma in situ)



Figs 23.3A to F: (A) Hematoxylin and Eosin (H and E); (B) Diagrammatic LSIL with koilocyte; (C) Cervical cytology showing koilocytes; (D) HSIL (H&E); (E) Low-grade squamous intraepithelial lesion (LSIL); (F) High-grade squamous intraepithelial lesion (HSIL). Cervical cytology with pleomorphism and nuclear atypia

INVASIVE CARCINOMA OF CERVIX

• Precursor lesion:

HSIL: Precursor of cervical squamous cell carcinoma.

- HSIL is an immediate precursor of **cervical squamous cell carcinoma**.
- Precursor lesion for adenocarcinoma is called adenocarcinoma in situ.
- **Age: Cervical cancer** is usually found between **40 and 60 years** (mean age 54 years) with peak at 45 years.

Etiology and Pathogenesis

(Refer Pages 203-204 and 658–660)

MORPHOLOGY

Q. Write short note on morphology of carcinoma of cervix.

Gross

Carcinoma cervix: Gross patterns

1. Fungating
2. Ulcerative
3. Infiltrative.

Carcinoma of cervix arise from the transformation zone. Three patterns of invasive cervical carcinoma are:

1. **Fungating (exophytic):** Forms a nodular and **exophytic cauliflower like** growth protruding into vagina (Fig. 23.4).
2. **Ulcerative lesion:** It appears as **ulcerated, granular, eroded** lesion.
3. **Infiltrative mass:** If the tumor is located within the **endocervical canal**, it may infiltrate the stroma and cause diffuse enlargement and hardening of the cervix (**barrel-shaped cervix**).

Carcinoma cervix: Squamous cell carcinoma is the most common histological subtype.

Microscopy

Carcinoma cervix: Most common cancer in women in India.

A. Squamous cell carcinoma:

- **Most common** (~ 80%) histological subtype of cervical cancer.
- Composed of **solid nests and groups of malignant squamous cells** (either keratinizing or nonkeratinizing) **invading the underlying stroma** of the cervix.
- **Subtypes:** Depending on the cytological features, it can be divided into **two subtypes**.

Fungating tumor as seen from external os of cervix

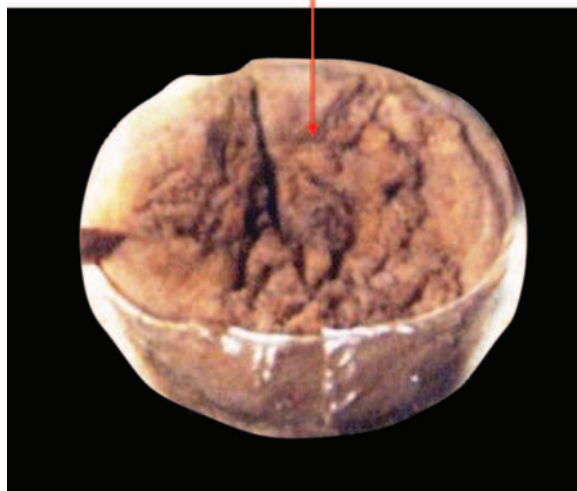


Fig. 23.4: Squamous cell carcinoma of cervix presenting as a fungating growth projecting from cervical os

Malignant cells invading the stroma Production of keratin by tumor cells

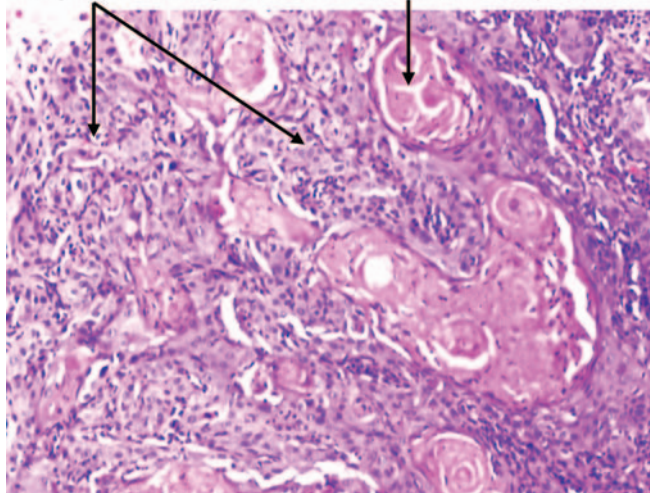


Fig. 23.5: Photomicrograph of invasive keratinizing squamous cell carcinoma of cervix

Microscopic types of squamous cell carcinoma of cervix:

1. Large cell keratinizing
2. Large cell non-keratinizing

1. **Large cell keratinizing:** It consists of nests of keratinized cells, which form concentric whorls known as **keratin or epithelial pearls** (Fig. 23.5).
2. **Large cell nonkeratinizing:** This pattern shows nests of large malignant squamous cells with individual cell keratinization but without any keratin pearls.

B. Adenocarcinoma:

- **Second most common** tumor type (~ 15%).
- **Microscopy** (Fig. 23.6):
 - Consists of **malignant endocervical cells forming glandular pattern**.
 - Tumor cells have large, hyperchromatic nuclei and with minimal mucin in the cytoplasm.

C. Adenosquamous carcinomas are rare cervical tumors.

- **Adenosquamous carcinoma:** It is composed of malignant glandular epithelium admixed with malignant squamous epithelium.

D. Neuroendocrine cervical carcinoma: It appears similar to small-cell carcinoma of the lung.

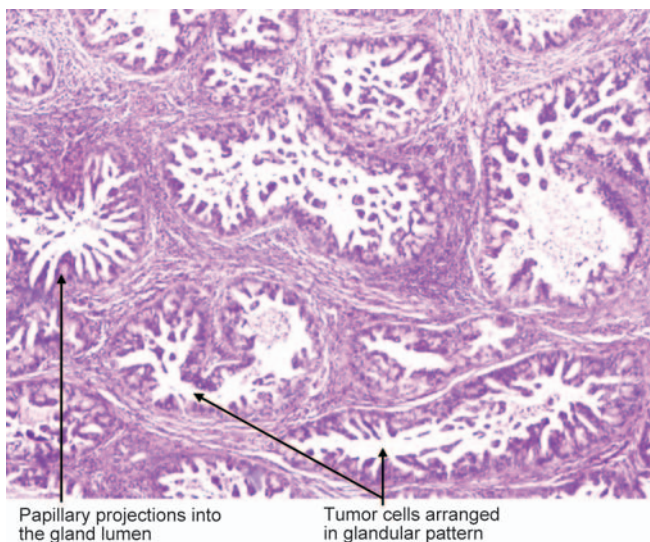


Fig. 23.6: Photomicrograph of adenocarcinoma of cervix

- **Ureters:** If involved may lead to its obstruction → hydroureter, hydronephrosis, pyelonephritis, and renal failure (uremia).
- **Vagina.**

2. **Lymphatics:** Tumor may spread to regional lymph nodes and involve paracervical, hypogastric, and external iliac nodes.
3. **Hematogenous spread:** Liver, lungs and bone marrow.

Spread of Carcinoma Cervix

Q. Write short note on spread of carcinoma of cervix.

1. **Direct extension:** Into the surrounding tissues and organs.
 - Paracervical tissues.
 - Urinary bladder and rectal: Their involvement may lead to fistula formation.

Clinical Features

- During early stages of cervical cancer, patients most often present with **vaginal bleeding** after intercourse.

- With more advanced tumors, symptoms depend on the route and degree of spread.

Prognosis for invasive carcinomas depends largely on the stage of the carcinoma.

Cervical Cancer Screening

Cervical cancer: Reduced incidence because of cytologic screening by Pap smear examination.

Q. Write short note on diagnosis of carcinoma of cervix.

Cytologic Screening by Pap Smear Examination

- Majority of cervical cancers are preceded by a precancerous lesion and **cytologic screening by Pap smear** is the **most reliable screening test** for preventing and also detecting noninvasive stage of cervical cancer.
- **Pap tests** are **cytological preparations of exfoliated cells from the cervix** that are **stained with the Papanicolaou method**. Using a spatula (Fig. 23.7A) or brush, the transformation zone of the cervix is circumferentially scraped and the cells are smeared onto a slide. Following fixation and staining, the smears are screened to **identify cytologic abnormalities** (Fig. 23.7B).

Pap smear: Highly effective for screening CIN and cervical cancer.

- In addition, **HPV DNA testing** may be carried out.

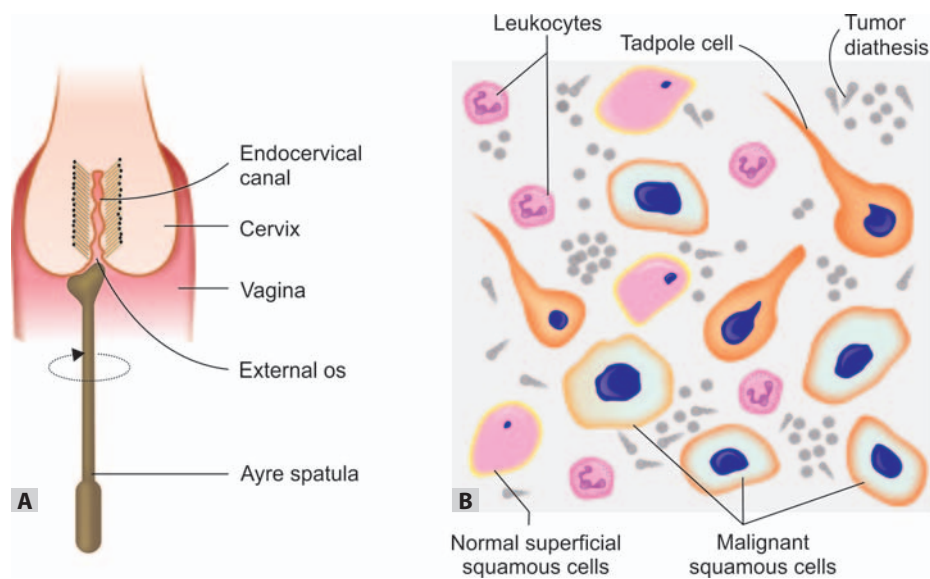
Histologic Diagnosis and Removal of Precancerous Lesions

- If the **Pap test** shows **abnormal cells**, a **colposcopic examination** of the cervix and vagina is performed to know the extent of the lesion and lesion is biopsied.
- Application of **acetic acid to the cervix highlights abnormal areas**.
- After confirmation by tissue biopsy, women with LSIL can be followed up with repeat smears.
- **HSILs are treated with cervical conization** (excision) and follow-up smears and clinical examinations for life.
- **Surgical removal of invasive cancers, with adjunctive therapy** (radiation and chemotherapy).

Cervical Cancer Prevention

Cervical Pap smear uses:

- For screening SIL and cervical cancer
- Evaluation of hormonal status.
- Prophylactic HPV vaccine for HPV types 6, 11, 16, and 18 has been used to reduce the incidence of cervical cancer.
- The vaccine is prepared from noninfectious, DNA-free virus-like particles produced by recombinant technology.
- It produces high levels of serum antibodies in vaccinated individuals.



Figs 23.7A and B: (A) Method of obtaining cervical smear by Ayre's spatula; (B) Cytological appearance of squamous cell carcinoma of cervix (diagrammatic)

UTERUS

MENSTRUAL CYCLE

The endometrium undergoes characteristic morphologic changes during the menstrual cycle due to sex hormones produced in the ovary.

The normal menstrual cycle begins with the shedding of the upper half to two-thirds of the endometrium during menses.

Proliferative Phase (Fig. 23.8)

Proliferative phase: Mediated by estrogen and most variable phase.

- **Endometrial glands:**
 - During the proliferative phase, the endometrial glands are **straight, tubular and evenly distributed**.
 - Glands are **lined by regular, tall, pseudostratified columnar cells**. Mitotic figures are numerous.
- **Endometrial stroma:**
 - Consists of **compactly arranged spindle cells** having scanty cytoplasm but abundant mitotic activity.
 - Spiral arteries are narrow and mostly inconspicuous.

Secretory Phase

Secretory phase: Mediated by progesterone and least variable phase.

- **Endometrial glands:**

Subnuclear vacuolation: Histological feature of ovulation.

- **Enlarge, dilate, and become more coiled.**
- The lining cells develop abundant and prominent, glycogen-rich, **subnuclear vacuoles** (Fig. 23.9).
- The **secretion increases** and the basal vacuoles progressively push past the nuclei. Later, the **secretions are discharged into the lumen** of the gland → the glands are dilated and tortuous, producing a serrated (“**saw-toothed**”) appearance (Fig. 23.10) when they are cut in their long axis.
- **Endometrial stroma:**
 - The stromal cells enlarge (hypertrophy) and show **large, round, vesicular nuclei and abundant eosinophilic cytoplasm** and reappearance of stromal mitoses.
 - These cells are the precursors of the decidual cells of pregnancy and are referred to **predecidual change**.

Menstrual Phase

- In the absence of pregnancy, spiral arteries collapse, and the disintegration of the functionalis begins.
- The blood escapes into the stroma, marking the beginning of menstrual shedding. Menses commence on day 28, lasts 3–7 days.

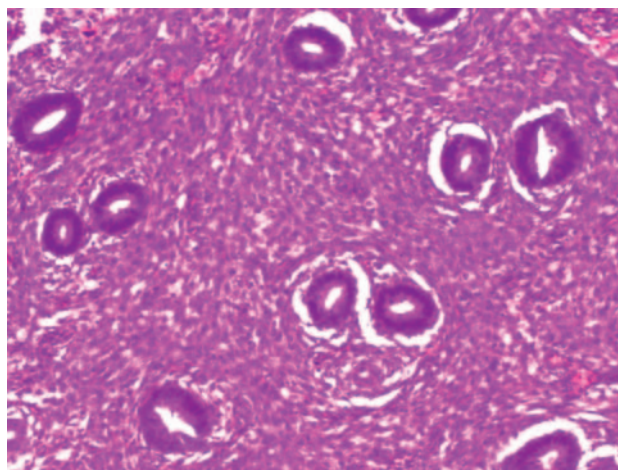


Fig. 23.8: Proliferative phase composed of tubular endometrial glands lined by tall columnar cells

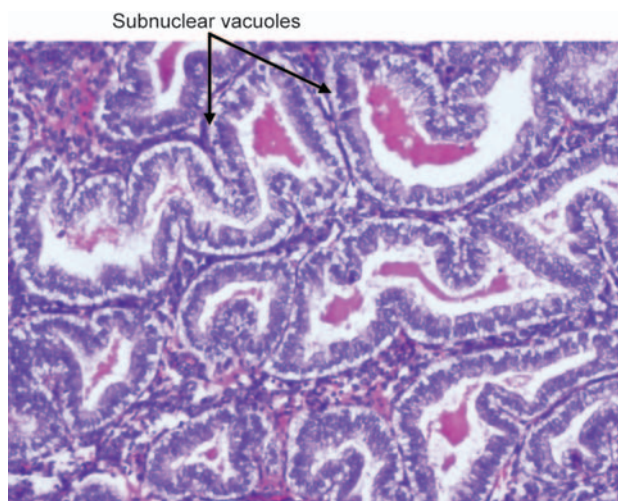


Fig. 23.9: Early secretory phase shows endometrial glands lined by tall columnar cells with subnuclear vacuoles

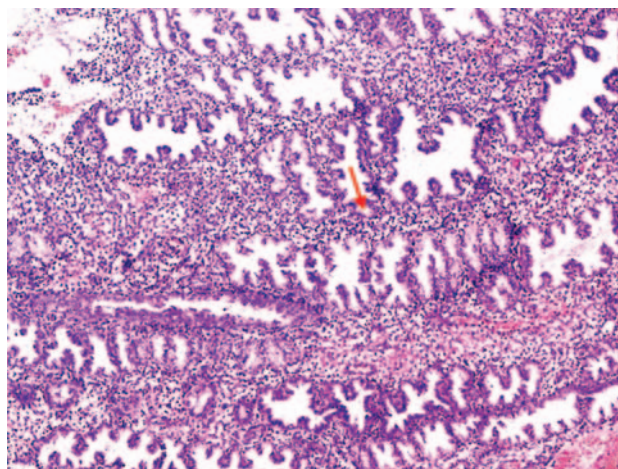


Fig. 23.10: Late secretory endometrium saw-tooth appearance of gland and predecidual changes in the stroma

ENDOMETRIOSIS

Q. Write short note on endometriosis.

Definition: Endometriosis is the **presence of endometrial tissue** (glands and stroma) **outside of the uterus**.

Endometriosis: Functioning endometrial glands and stroma outside the uterus.

Age: Mainly occurs **during active reproductive life**, mostly in **third and fourth decades**.

Incidence: Affect ~ 5–10% of women.

Pathogenesis

The proposed origin of endometriosis may be from two main sources: (1) from the uterine endometrium and (2) from outside the uterus from cells which has the capacity to give rise to endometrial tissue. Main theories includes:

1. **Regurgitation theory:** According to this, the endometrial tissue by retrograde menstruation passes through the fallopian tubes and implanted at abnormal/ectopic site. It can explain the distribution of endometriosis within the peritoneal cavity. Retrograde menstruation occurs even in normal women.
2. **Benign metastases theory:** According to this, endometrial tissue can spread from the uterus to distant sites (e.g. bone, lung, and brain) via blood vessels and lymphatic channels.
3. **Metaplastic theory:** According to this theory, endometrium arises directly from metaplasia of celomic epithelium (mesothelium of pelvis or abdomen). During embryonic development, the Müllerian ducts and the endometrium arise from celomic epithelium.
4. **Extrauterine stem/progenitor cell theory:** It is a recent theory according to which the endometrial tissue in ectopic site represents differentiation of stem/progenitor cells from the bone marrow.

Molecular changes: Molecular analysis of endometriotic tissue showed some differences when compared to the endometria in a women without endometriosis. These include:

- **Release of proinflammatory and other factors**, e.g. PGE₂, IL-1 β , TNF α , IL-6 and -8, etc.
- **Increased estrogen production by endometriotic stromal cells.**
- **Association between endometriosis and ovarian endometrioid and clear cell type carcinoma** is observed. Shared mutations in specific genes (PTEN and ARID1A) is found in endometriotic cysts, atypical endometriosis and associated carcinomas.

MORPHOLOGY

- **Sites** (Table 23.2).

Gross

- They produce **nodules** with a **red-blue to yellow-brown** appearance at the site of endometriosis.
- Foci of endometriosis **respond to both extrinsic and intrinsic hormonal stimulation** with **periodic bleeding** \rightarrow **organization of hemorrhage** causes fibrosis \rightarrow subsequent **fibrous adhesions** between neighboring structures (e.g. tubes, ovaries and obliterates the pouch of Douglas).
- **Endometriosis of ovary:**

Q. Write short note on endometriosis/chocolate cyst of ovary.

Endometriosis of ovary: Chocolate cysts.

Endometriosis of ovary: Most common site \rightarrow chocolate cysts.

- Ovaries may be distorted by numerous **cystic spaces** (1 to 5 cm in diameter) **filled with dark brown fluid** (due to previous hemorrhage).
- **Repeated hemorrhage** may form **large cysts** up to 15 cm in diameter, which **contain inspissated, chocolate-colored material** \rightarrow clinically known as **chocolate cysts** or endometriomas.

Microscopy

Endometriosis: Extrauterine

- Endometrial glands
- Stroma.
- Commonly, shows **both endometrial glands and stroma with or without** the presence of **hemosiderin-laden macrophages** at the ectopic site.
- Rarely, may show only endometrial stroma and/or hemosiderin-laden macrophages.

Clinical Features

Endometriosis triad:

1. Dysmenorrhea
2. Dyspareunia
3. Infertility.

BOX 23.2: Various sites of endometriosis in descending order of frequency

1. Ovaries (more than 60%)
2. Uterine ligaments
3. Rectovaginal septum
4. Cul de sac
5. Pelvic peritoneum
6. Large and small bowel and appendix
7. Cervix, vagina, and fallopian tubes
8. Laparotomy hysterectomy scars

- Dysmenorrheal (painful menstruation).
- Dyspareunia (pain during intercourse).
- Pelvic pain (due to the intrapelvic bleeding and periuterine adhesions).
- Pain on defecation (rectal wall involvement).
- Dysuria (involvement of serosa of the bladder).
- Intestinal disturbances (when the small intestine is affected).
- Menstrual irregularities.

Endometriosis: Undergoes cyclic bleeding.

Consequences

- Infertility in ~ 30–40% of women.
- Malignancy: Uncommon.

ADENOMYOSIS

Q. Write short note on adenomyosis of uterus.

Definition: Adenomyosis is defined as the **presence of endometrial tissue within the myometrium** (uterine wall).

Adenomyosis seen in ~ 20% of uterine specimens.

Adenomyosis: Endometrial glands and stroma within the myometrium.

Etiology and Pathogenesis

Pathogenesis is **not known**. Adenomyosis is seen in continuation with the endometrium. So, it may develop due to down growth followed by proliferation of endometrial tissue into and between the smooth muscle fascicles of the underlying myometrium.

MORPHOLOGY

Gross

- Uterus may show **mild to moderate enlargement**.
- Cut section of the **uterine wall** shows coarse **trabeculations** with **ill-defined area of hemorrhage**.

Microscopy (Fig. 23.11)

- Shows **irregular nests of benign endometrial glands and stroma** deep within the **myometrium**.
- The **minimum distance between the basal endometrium and the endometrial tissue** of adenomyosis should be **one low power microscopic field** (i.e. at least 2–3 mm).

Clinical Features

Adenomyosis: No cyclic bleeding.

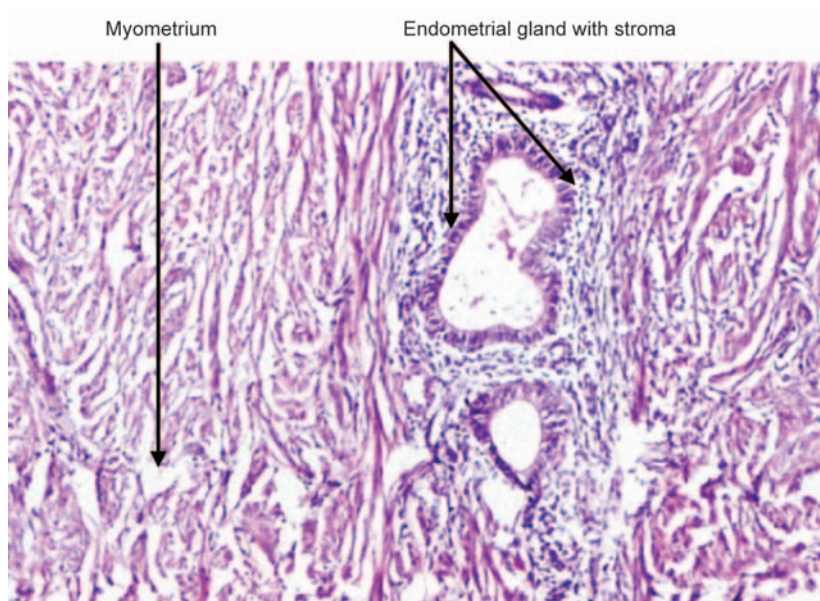


Fig. 23.11: Adenomyosis showing nests of endometrial glands and stroma deep within the myometrium

- Metrorrhagia (irregular and heavy menses).
- Colicky dysmenorrhea.
- Dyspareunia.
- Pelvic pain (during premenstrual period).

WHO (2014) Classification of Tumors of Uterine Corpus (Box 23.3)

BOX 23.3: WHO (2014) Classification of tumors of uterine corpus (abridged)

Epithelial tumors and precursors

• Precursors

- Hyperplasia without atypia
- Atypical hyperplasia/endometrioid intraepithelial neoplasia

• Endometrial carcinoma

- Endometrioid carcinoma (type I)
- Serous carcinoma (type II)
- Neuroendocrine tumors
 - ◆ Low-grade neuroendocrine tumor: Carcinoid tumor
 - ◆ High-grade neuroendocrine carcinoma
 - ◇ Small cell neuroendocrine carcinoma (formerly termed small cell carcinoma)
 - ◇ Large cell neuroendocrine carcinoma

Mesenchymal tumors

- Benign: Leiomyoma
- Malignant: Leiomyosarcoma

Other tumors

ENDOMETRIAL HYPERPLASIA

Q. Write short note on endometrial hyperplasia/causes of endometrial hyperplasia.

Definition: Endometrial hyperplasia is defined as **increased thickness of endometrium** due to an **increased proliferation of the endometrial glands relative to the stroma**. This results in an increased gland-to-stroma ratio when compared with normal proliferative endometrium.

- Endometrial hyperplasia is an important **cause of abnormal uterine bleeding**.
- **Soil for carcinoma:** Endometrial hyperplasia is a soil for endometrial carcinoma and both share specific molecular genetic alterations.

Endometrial hyperplasia: Increased thickness of endometrium due to increased proliferation of endometrial glands.

Endometrial hyperplasia: Soil for endometrial carcinoma.

Etiology

Endometrial hyperplasia: Prolonged estrogen stimulation.

1. **Prolonged estrogen stimulation:** It can lead to endometrial hyperplasia. Increased estrogen may be due to:

• Increased estrogen production

– Endogenous sources

- ◆ **Obesity:** It is associated with increased peripheral conversion of androstenedione to estrone by the enzyme aromatase in fat cells.
- ◆ **Polycystic ovarian disease, e.g. Stein-Leventhal syndrome.**
- ◆ **Functioning granulosa cell tumors** of the ovary.
- ◆ **Excessive cortical function, e.g. cortical stromal hyperplasia.**

– Exogenous estrogen: Prolonged administration of estrogen (e.g. estrogen replacement therapy).

• Anovulation, e.g. menopause.

2. **Mutation of tumor suppressor gene *PTEN*:** It is seen in both endometrial hyperplasias and carcinomas.

- Loss of PTEN function may **activate cellular pathways** normally activated by estrogen.
- Thus, inactivation of the PTEN and hyperestrogen may act in conjugation both in endometrial hyperplasia and endometrial carcinoma.

Endometrial hyperplasia: Diffuse process involving the entire endometrium.

Mutation of *PTEN* (tumor suppressor) gene: Seen in both hyperplasia and carcinoma of the endometrium.

Classification

Over the years, the classification of endometrial hyperplasia has undergone a number of changes.

Old Classification

It was most widely used and was based on architectural and cytologic features. It consisted of four categories:

1. **Simple hyperplasia without atypia:** Also known as **cystic or mild hyperplasia** and is usually due to persistent estrogen stimulation. Progression to adenocarcinoma is uncommon (~ 1%). Microscopically, it shows mild increase in the gland-to-stroma ratio. The glands are of various sizes and irregular shapes with cystic dilatation (Figs 23.12A and B) → results in a **Swiss-cheese** appearance.
2. **Simple hyperplasia with atypia:** Uncommon and its progression to adenocarcinoma is about ~8%. Microscopically (Fig. 23.13A), it appears like simple hyperplasia, but glandular epithelial cells show cytologic atypia.

3. **Complex hyperplasia without atypia:** Only ~ 3% progress to adenocarcinoma which is lower than that of simple hyperplasia with atypia. Microscopically (Fig. 23.12C), number and size of endometrial glands increased with marked gland crowding: (back-to-back with little intervening stroma), branching of glands and abundant mitotic figures. Epithelial cells are cytologically normal.
4. **Complex hyperplasia with atypia** (Figs 23.13B and C): It **morphologically overlaps** with well-differentiated endometrioid adenocarcinoma.

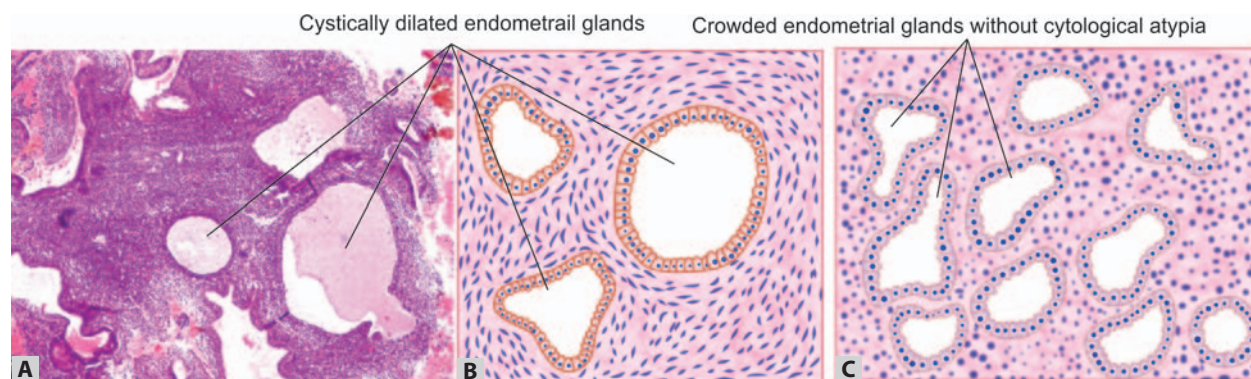
World Health Organization (WHO) 2014 Classification

The most current classification divides endometrial hyperplasia into two major categories:

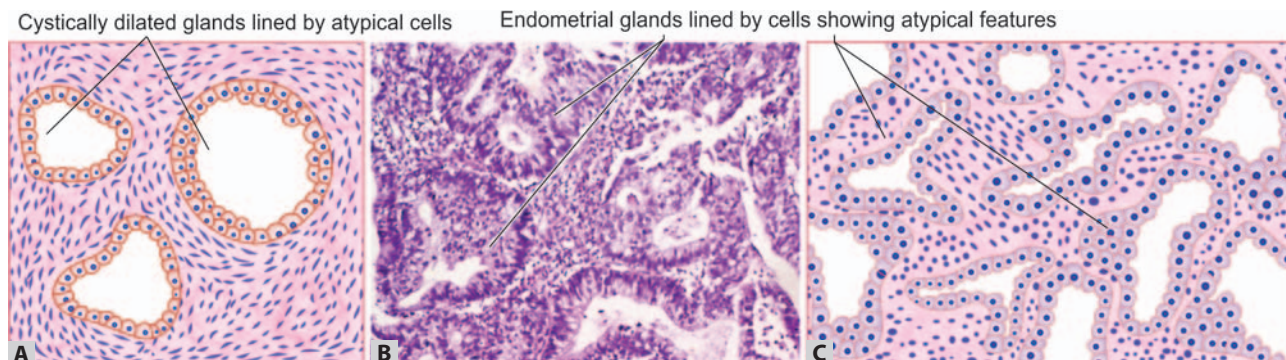
1. **Hyperplasia without atypia/Non-atypical hyperplasia** (Fig. 23.12): This is also termed hyperplasia without atypia and includes simple hyperplasia without

atypia and complex hyperplasia without atypia. Main feature is an increase in the gland-to-stroma ratio. The glands show variation in size and shape and may show dilatation but without significant cytological atypia. There may be focal back-to-back arrangement of glands with some intervening stroma. They are due to persistent estrogen stimulation and rarely progress to adenocarcinoma (~1% to 3%). It may undergo cystic atrophy when estrogen is withdrawn.

2. **Atypical hyperplasia/Endometrioid intraepithelial neoplasia** (Fig. 23.13): This includes simple hyperplasia with atypia, complex hyperplasia with atypia and endometrial intraepithelial neoplasia. It consists of complex patterns of proliferating glands with nuclear atypia. The glands are usually crowded show back-to-back arrangement and often with complex outlines due to branching of glands and loss of polarity. The nuclei are enlarged, pleomorphic and consist of open (vesicular) chromatin and conspicuous nucleoli. These features may overlap with those of well-differentiated



Figs 23.12A to C: Endometrial hyperplasia without atypia. (A) hematoxylin and Eosin; (B) (Diagrammatic) Old terminology- Simple hyperplasia without atypia showing cystic dilatation of glands; (C) (Diagrammatic) Old terminology-complex hyperplasia without atypia



Figs 23.13A to C: Atypical endometrial hyperplasia; (A) Diagrammatic appearance Old terminology-simple hyperplasia with atypia; (B) Hematoxylin and Eosin; (C) (Diagrammatic) Old terminology-complex hyperplasia with atypia

endometrioid adenocarcinoma. Differentiating atypical hyperplasia from endometrial cancer may not be possible without hysterectomy.

CARCINOMA OF THE ENDOMETRIUM

- Carcinoma of the endometrium is the most common pelvic invasive cancer of the female genital tract.
- **Age group:** Carcinoma of the endometrium is uncommon in before 40 years of age, and mainly occur in **postmenopausal women between 55 and 65 years**.
- Clinically present as **abnormal (postmenopausal) bleeding**.

Molecular Pathogenesis

Classification: According to **clinicopathological and molecular features** carcinoma of the endometrium is classified into two types namely type I and type II (Table 23.1).

Type I Carcinomas (Endometrioid Cancers)

- **Most common type** (>80%).
- **Majority** of them are **well-differentiated** and mimic proliferative endometrial glands → known as **endometrioid carcinoma**. Develops from precursor of **endometrial hyperplasia**.
- Associated with: (1) obesity, (2) diabetes, (3) hypertension, (4) infertility (nulliparous or have anovulatory cycles), and (5) unopposed estrogen stimulation.

Molecular Changes

Endometrial carcinoma develops in a stepwise pattern by acquiring several genetic alterations in tumor suppressor genes and oncogenes. The **hallmark** of type I endometrioid carcinoma is that the **most common mutations increase**

signaling through the PI3K/AKT pathway. It is observed that individual tumor may have many mutations that increase PI3K/AKT signaling. This suggests that tumor development and progression is due to successive increases in signal strength. Mutations that increase PI3K/AKT signaling in endometrial carcinomas are:

- Mutations in the *PTEN* tumor suppressor gene (>50%).
- Mutations in *PIK3CA* (oncogene that encodes the catalytic subunit of PI3K) (~30%) and play a role in invasion.
- *KRAS* mutations (~25%).
- Loss-of-function mutations in *ARID1A* (a regulator of chromatin structure) (40% low-grade carcinoma).
- Defects involving *DNA mismatch repair genes* and microsatellite instability~35%.
- Loss-of-function mutations in *TP53* (~30%)

MORPHOLOGY

- **Gross** (Fig. 23.14): Endometrial carcinoma may be:

Gross appearance of type I endometrial carcinoma:

1. Diffuse
2. Local polypoid.

- **Diffuse tumor** involving the endometrial surface
- **Localized polypoid one or more discrete nodules**.

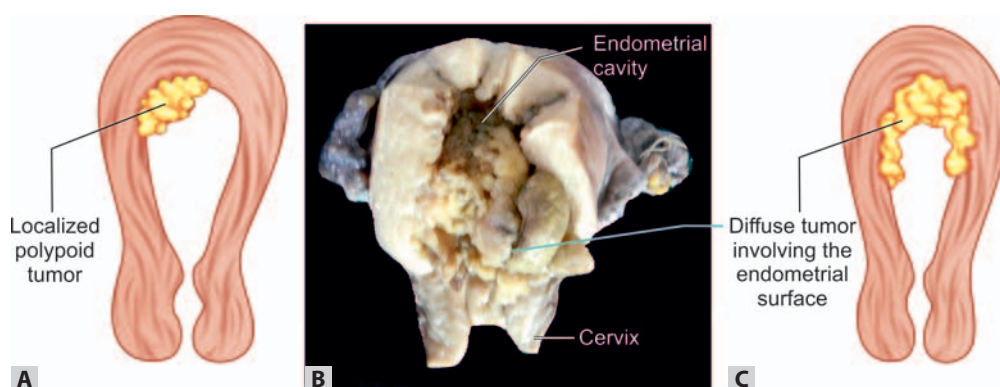
Large tumors usually show areas of hemorrhage and necrosis.

- **Microscopy:**

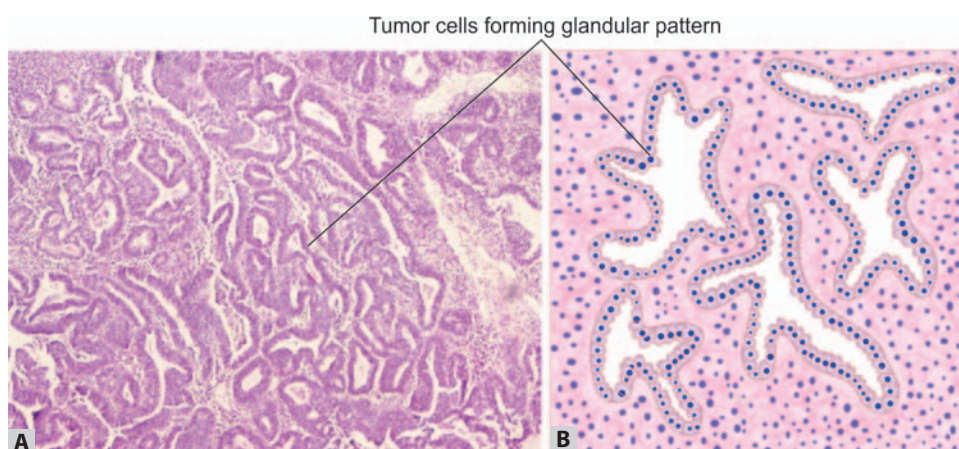
- Most endometrial carcinomas (~85%) are **endometrioid adenocarcinomas** (Fig. 23.15).
- **Glandular pattern:** Resembles normal endometrial epithelium
- **Nuclei:** Range from **bland to markedly pleomorphic**
- **Nucleoli:** **Prominent**.
- **Mitotic figures:** **Abundant** and may show abnormal mitotic figures in less differentiated tumors.

TABLE 23.1: Differentiating features of type I and type II endometrial carcinoma

Characteristics	Type I	Type II
Age	55–65 years	65–75 years
Predisposing conditions	Unopposed estrogen stimulation, obesity, hypertension, infertility, diabetes	Atrophy of endometrium Thin physique
Precursor lesion	Atypical endometrial hyperplasia	Serous endometrial intraepithelial carcinoma
Molecular features	Mutations or inactivation of <i>PTEN</i> , mutations in <i>PIK3CA</i> , <i>PIK3R1</i> , <i>ARID1A</i> , <i>KRAS</i> , and <i>TP53</i> . Microsatellite instability	Mutations in <i>TP53</i> , <i>PIK3CA</i> , <i>FBXW7</i> , <i>PPP2R1A</i> . Germline <i>BRCA1/2</i> mutations
Microscopy	Endometrioid adenocarcinoma ranges from well to poorly differentiated	<ul style="list-style-type: none"> • Serous • Clear cell • Mixed Müllerian tumor
Spread	Via lymphatics	Intraperitoneal and via lymphatic
Behavior	Indolent	Aggressive



Figs 23.14A to C: Endometrioid adenocarcinoma of endometrium. (A) Localized polypoid tumor; (B) Gross specimen; (C) Diagrammatic. Diffuse tumor involving the endometrial surface



Figs 23.15A and B: Microscopic appearance of endometrial adenocarcinoma. (A) Hematoxylin and eosin (H and E); (B) Diagrammatic

- **Grading:** It is done according to the glandular differentiation alone.
 - **Well-differentiated (grade 1/G1) adenocarcinoma:** It consists of tumor cells forming **easily recognizable glandular patterns**.
 - **Moderately-differentiated (grade 2/G2) adenocarcinoma:** It consists of mixture of both **well-formed glands and solid sheets** of malignant cells.
 - **Poorly-differentiated (grade 3/G3) adenocarcinoma:** It consists of **solid sheets (more than 50%)** of cells **without recognizable glands**. They show severe degree of nuclear atypia and numerous mitotic figures.
- **Serous adenocarcinoma** is the most common subtype and similar to serous adenocarcinoma of the ovary. It may show papillary projections lined by columnar cells with moderate nuclear atypia similar to ovarian papillary serous carcinoma (refer Fig. 23.21C).
- Less common histological subtypes:
 - Clear cell adenocarcinoma, carcinosarcoma (malignant mixed Müllerian tumor).

Molecular Changes

Most common mutations include *TP53*, *PIK3CA*, *FBXW7* and *PPP2R1A* and germline *BRCA1/2* mutations (Table 23.2). Serous carcinoma probably begins as a surface epithelial neoplasm that extends into adjacent gland structures and later invades endometrial stroma.

MORPHOLOGY

Serous carcinomas usually arise in small atrophic uteri. They form large bulky mass or deeply invade the myometrium.

Type II Serous Carcinomas

- **Age:** Usually occur a decade later than type I carcinoma.
- **Poorly differentiated (grade 3) tumors.** Constitute ~15% of endometrial carcinoma. Associated with **bad prognosis**. Usually arise in the **background of endometrial atrophy**.

- **Serous endometrial intraepithelial carcinoma (SEIC)** is the precursor lesion **that arises from a polyp or atrophic endometrium**. The lesion is confined to the endometrium and consists of malignant cells similar to serous carcinoma that arises from the epithelial surfaces.
- **Invasive serous carcinomas** may have a papillary growth pattern and consists of cells with marked cytological atypia (high nuclear to cytoplasmic ratio, atypical mitotic figures, hyperchromasia, and prominent nucleoli). However, they may have a glandular growth pattern which is differentiated from endometrioid carcinoma by the presence of marked cytological atypia. Irrespective of histological pattern, all these tumors are classified as grade 3. Serous carcinoma with relatively superficial endometrial involvement shed tumor cells and extensively metastasize to peritoneal surfaces and extrauterine sites by routes (i.e. tubal or lymphatic transmission) other than direct invasion.

Clinical Features of Endometrial Carcinoma

- **Age:** Mostly seen in postmenopausal women between 55 and 65 years of age.
- **Irregular or postmenopausal vaginal bleeding** with excessive leukorrhea.
- **Diagnosis** by histologic examination of tissue obtained by biopsy or curettage.

Spread

- **Direct invasion:** The tumor can invade the underlying myometrium and may extend into the periuterine structures.
- **Lymphatic spread:** To the regional lymph nodes. Serous carcinoma spreads to extrauterine (lymphatic or transtubal) site, even when it is confined to the endometrium or its surface epithelium.
- **Hematogenous spread:** To the lungs, liver, bones, and other organs.

Malignant Mixed Müllerian Tumors (MMMTs)

Also termed as **carcinosarcomas** are **endometrial adenocarcinomas with a malignant mesenchymal component**. They resemble endometrial carcinoma genetically and have poor prognosis. Microscopically, they usually consist of adenocarcinoma (endometrioid, serous, or clear cell) along with the malignant mesenchymal (sarcomatous) component.

LEIOMYOMAS

Uterine leiomyoma: Most common benign tumor of uterus.

Uterine leiomyomas (commonly called **fibroids**) are **benign smooth muscle neoplasms** and are **most common tumor in females**.

Molecular Changes

Majority of leiomyoma have normal karyotypes, but about 40% may have a simple chromosomal abnormality. These include (i) rearrangements of chromosomes 12q14 and 6p involving the *HMGIC* and *HMGIIY* genes, respectively and (ii) mutations in the *MED12* gene.

MORPHOLOGY

Gross

- Leiomyomas are **sharply circumscribed** (without encapsulation), **discrete, round, firm, gray-white** tumors.
- **Size:** Vary in size from small nodules to massive tumors, which fill the pelvis.
- **Number:** May be single or multiple.
- **Cut section:** It has a characteristic **whorled pattern** of smooth muscle bundles and has a **raw (watered) silk appearance**.
- **Red degeneration:** It may be **observed in large tumors** and is characterized grossly by a bulging surface and a **homogeneous dark brown to red appearance on cut section**.
- **Sites (Fig. 23.16):**
 - **Most common:**
 - ♦ **Within the myometrium** (intramural) of the corpus.
 - ♦ **Just beneath the endometrium** (submucosal).
 - ♦ **Beneath the serosa** (subserosal) or pedunculated.
 - **Rare sites:** Uterine ligaments, lower uterine segment or cervix.

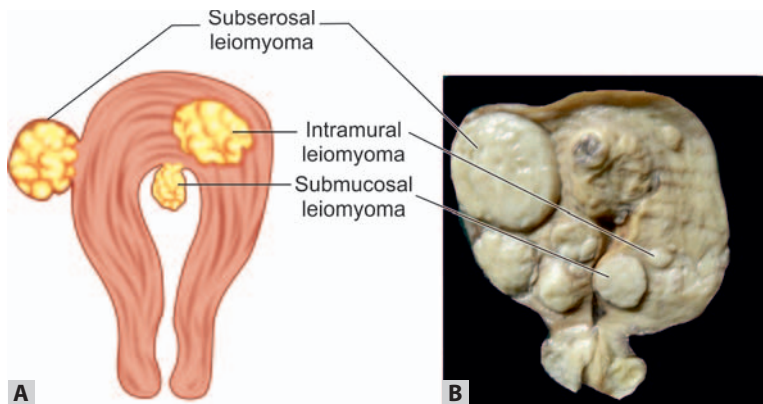
Leiomyoma-sites:

1. Intramural
2. Submucosal
3. Subserosal.

Microscopy (Fig. 23.17)

Leiomyoma: Interlacing bundles of smooth muscle cells.

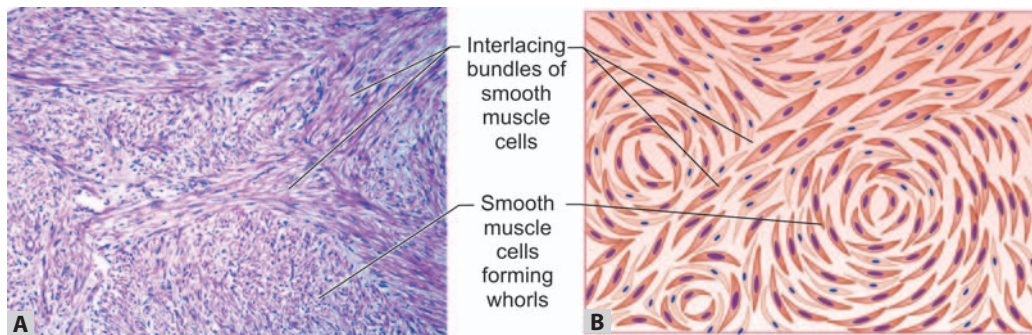
- **Pattern:**
 - Composed of **interlacing fascicles/whorled bundles of smooth muscle cells** (identical to the smooth muscle cells of the uninvolved adjacent myometrium).
 - Form circumscribed nodules and have increased cellularity that helps in distinguishing them from the normal myometrium.



Leiomyomas usually grow slowly, but occasionally enlarge rapidly during pregnancy.

Mitoses: Most important criteria in assessing malignancy in smooth-muscle tumors of the uterus.

Figs 23.16A and B: (A) Diagrammatic; (B) Gross specimen, shows different sites of leiomyoma



Figs 23.17A and B: Microscopic appearance of leiomyoma showing well-differentiated, regular, spindle-shaped smooth muscle cells arranged in interlacing fascicles. (A) Hematoxylin and eosin (H and E); (B) Diagrammatic

- **Tumor cells:**
 - Individual muscle **tumor cells are uniform in size and shape.**
 - **Nuclei:** They are **elongated oval with blunt ends.**
 - **Cytoplasm:** It is abundant, eosinophilic with long, slender bipolar cytoplasmic processes.
 - **Mitotic figures:** Usually not seen.
- **Secondary changes:**
 - **Hyaline change** (degeneration—refer Fig. 1.29), mucoid or **myxomatous** degeneration, calcification, cystic changes and fatty metamorphosis.
 - **Red degeneration:** It shows **extensive coagulative necrosis** and may be **associated with pregnancy** or the use of **contraceptive drugs.**

Secondary changes in leiomyoma:

1. Hyaline change
2. Myxomatous change
3. Calcification
4. Cystic change
5. Fatty metamorphosis
6. Red degeneration.

Red degeneration:

- Occurs in large tumors
- Grossly bulging surface
- Cut section—homogeneous dark brown to red
- Extensive coagulative necrosis
- Associated with pregnancy/oral contraceptive.

Clinical Features

- **Age group:** Rare before age 20 and most regress after menopause.
- Leiomyomas of the uterus may be asymptomatic.
- **Common symptoms:**
 - **Abnormal bleeding**
 - **Urinary frequency** (compression of the bladder)
 - **Infertility.**

Malignant transformation (leiomyosarcoma) is extremely rare.

OVARIES

OVARIAN TUMORS

Incidence: Ovarian cancer constitutes the **third most common female genital tract cancers**, the incidence of which is below only carcinoma of the cervix and the endometrium.

WHO Classification of Ovarian Neoplasms

Q. Classify ovarian tumors.

- Classification is **according to the tissue of origin** (Box 23.4 and Fig. 23.18).

Primary ovarian tumors: May arise from surface epithelium, germ cells or sex cord-stromal cells.

- **Primary tumors** may arise from one of three ovarian components:
 - ◆ Surface epithelium which is derived from the celomic epithelium.
 - ◆ Germ cells which migrate to the ovary from the yolk sac.
 - ◆ Sex cord/stroma of the ovary.
- **Secondary or metastatic tumors.**

TUMORS OF SURFACE (MÜLLERIAN) EPITHELIUM

Q. Describe the morphological features of surface epithelial tumors.

Surface epithelial tumors: Most common primary neoplasms in the ovary.

- Surface epithelial tumors are the **most important and common primary** neoplasms in the ovary.
- **Origin:** They arise from the surface, celomic, or germinal epithelium which the outer aspect of the ovary. Most primary ovarian tumors arise from Müllerian epithelium.

Classification

Surface epithelial tumors: Most common malignant ovarian tumors and are seen in women older than 40 years of age.

Depending on the following features:

BOX 23.4: WHO (2014) classification of ovarian neoplasms (abridged)

A. PRIMARY TUMORS

1. Surface epithelial tumors

- Serous tumors
 - Benign (cystadenoma, cystadenofibroma)
 - Borderline (serous borderline tumor)
 - Malignant (low- and high-grade serous adenocarcinoma)
- Mucinous tumors, endocervical-like and intestinal type
 - Benign (cystadenoma, adenofibroma)
 - Borderline (mucinous borderline tumor)
 - Malignant (mucinous carcinoma)
- Endometrioid tumors
 - Benign (cystadenoma, adenofibroma)
 - Borderline (endometrioid borderline tumor)
 - Malignant (endometrioid carcinoma)
- Clear cell tumors
 - Benign (cystadenoma, adenofibroma)
 - Borderline (clear cell borderline tumor)
 - Malignant (clear cell carcinoma)
- Brenner tumors
 - Benign Brenner tumor
 - Borderline Brenner tumor/atypical proliferative Brenner tumor
 - Malignant Brenner tumor
- Seromucinous tumors: Benign, borderline and malignant

2. Germ cell tumors

- Mature teratoma: Cystic or solid
- Immature teratoma
- Monodermal teratoma and somatic-type tumors arising from dermoid cyst (e.g. struma ovarii, carcinoid)
- Dysgerminoma
- Yolk sac tumor (endodermal sinus tumor)
- Embryonal carcinoma
- Non-gestational choriocarcinoma
- Mixed germ cell tumors

3. Sex cord-stromal tumors

- Pure stromal tumors
 - Fibroma
 - Cellular fibroma
 - Thecoma
 - Leydig cell tumor
- Pure sex cord tumors
 - Adult granulosa cell tumor
 - Juvenile granulosa cell tumor
 - Sertoli cell tumor

B. SECONDARY TUMORS FROM NONOVARIAN PRIMARY

- Colonic, appendiceal, gastric, pancreaticobiliary, breast.

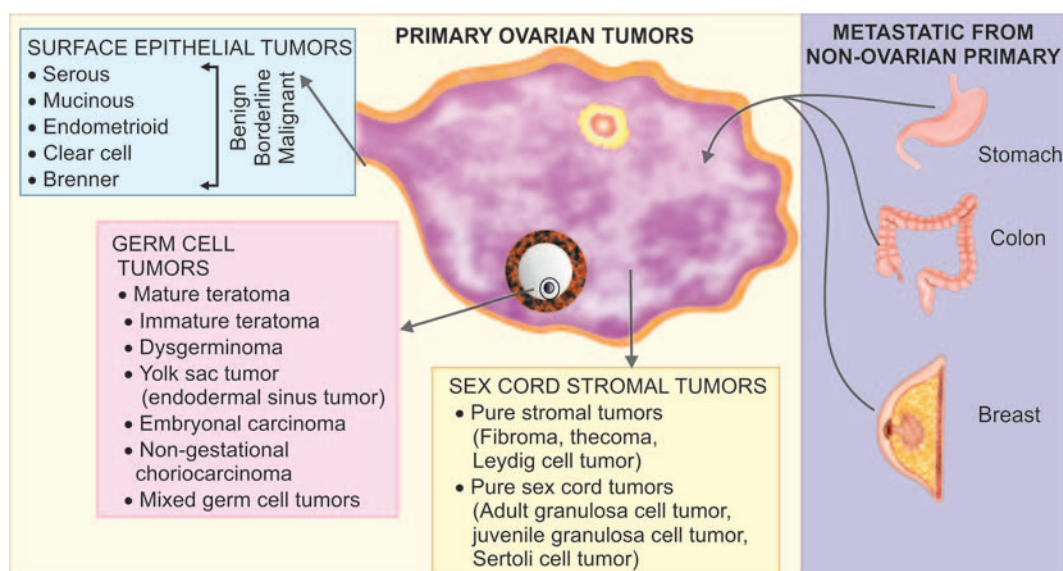


Fig. 23.18: Classification of ovarian neoplasms

Histological Types

Three major histological types are:

1. Serous
2. Mucinous
3. Endometrioid.

Biological Behavior

It is dependent on the degree of proliferation of the lining epithelium.

1. **Benign**
 - Epithelial proliferation is minimal.
 - ~ 80% are benign.
 - Mostly seen in young women between the ages of 20 and 45 years.
2. **Borderline** (also called atypical proliferative)
 - Shows moderate epithelial proliferation.
 - Occur at slightly older age.
3. **Malignant**
 - Shows marked epithelial proliferation with stromal invasion.
 - More common in older women, between 45 and 65 years.

Surface epithelial tumors:

- Benign
- Borderline
- Malignant subtypes under each type.

Pattern of Growth and Amount of Fibrous Stroma

The patterns include cystic, solid or those arise on the surface of ovary.

1. **Benign tumors**
 - Cystic areas: Cystadenomas
 - Cystic and fibrous areas: Cystadenofibromas
 - Predominantly fibrous areas: Adenofibromas
 - Surface papillary tumors.
2. **Borderline tumors and malignant tumors:** They can also have a cystic component, and when malignant they are referred to as cystadenocarcinomas.

Serous Tumors

Serous carcinomas: Most common malignant ovarian tumor and account for 40–50% of all cancers of the ovary.

Incidence: Serous tumors account for about 30% of all ovarian tumors and about over 50% of ovarian epithelial tumors.

- About 70% are benign or borderline. About 30% are malignant.

Age Group

- **Benign and borderline tumors:** Most common between 20 and 45 years.
- **Serous carcinomas:** Occur later in life.

Molecular Pathogenesis

- Risk factors for benign and borderline tumors are unknown.
- Risk factors for malignant serous tumors (serous carcinomas):
 - **Parity:** Nulliparous and women with low parity have higher risk.
 - **Gonadal dysgenesis** in children is associated with a higher risk.
 - **Genetic factors:** Heritable mutations in tumor suppressor genes *BRCA1* and *BRCA2* increase susceptibility to ovarian cancer.
 - **Family history** is a risk factor.
 - **Oral contraceptives and sterilization:** During reproductive period reduces the risk.

Serous carcinoma of ovary:

- Large, solid and cystic areas
- Multilayered epithelium
- Invasion.

Bilaterality in serous tumors of ovary:

- Benign ~20%
- Borderline ~30%
- Malignant ~66%.

Serous tumors—gross:

- Benign: Unilocular cyst
- Borderline: Predominantly cystic
- Malignant: Solid and cystic.

MORPHOLOGY

Q. Describe the morphological features of serous tumors of ovary.

Gross

- **Benign serous cystadenoma** (Fig. 23.19)
 - **Size:** Varies and may measure from 15 to 30 cm in diameter.
 - **Appearance:** It contains one or more thin-walled cysts. The cyst wall is **smooth glistening** without any epithelial thickening.
 - **Content:** Lumen filled with **clear watery serous fluid**.
 - **Cut section:**
 - ◆ Shows **smooth internal surface** and may show small **papillae** projecting into the cavity.
 - ◆ Rarely papillae seen projecting from the outer surface of ovary (surface papilloma).
 - **Bilaterality** is common and found in about 20% of benign serous cystadenomas.

Serous cystadenoma of ovary:

- Unilocular
- Bilateral common
- May show papillae.

• Borderline tumors:

- Vary in size, usually >5 cm and typically cystic.
- Cut section shows **cystic cavities** filled by increased number of **dense and closely packed, cauliflower-like papillary** projections.
- **Bilaterality:** 30%.

• Malignant serous adenocarcinoma (Fig. 23.20):

- Usually large.
- Shows a **mixture of solid and cystic areas** with large **solid or papillary areas**. Other features of malignancy includes: tumor irregularity, and fixation or nodularity of the capsule.

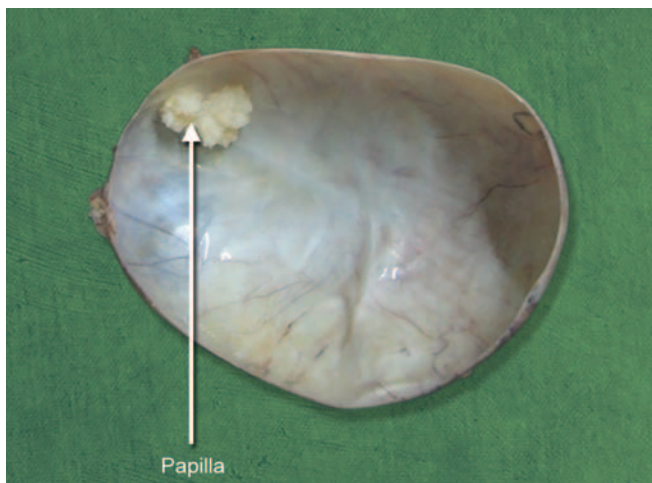


Fig. 23.19: Serous cystadenoma of ovary. Cut section shows uniloculated cyst with a focus of papilla

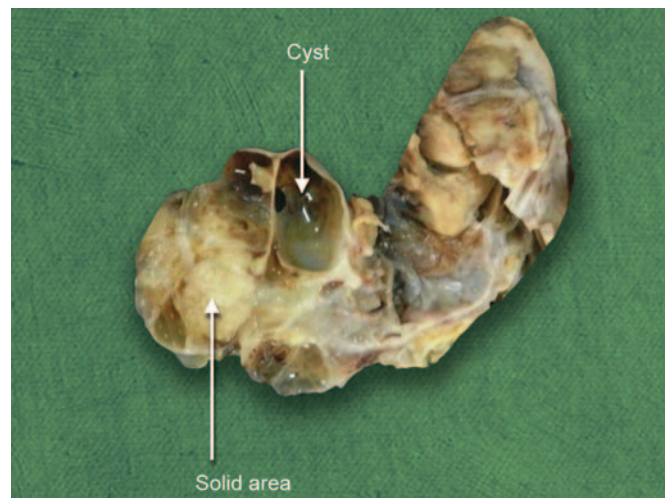


Fig. 23.20: Serous adenocarcinoma of ovary. Cut section only part of the tumor shows mixture of solid and cystic areas

- Areas of **necrosis and hemorrhage** in the solid area of tumor.
- **Adhesion to adjacent structures** (bowel, uterus and pelvic side wall).
- **Bilateral**: ~ 66%.

Microscopy

- **Benign serous tumors** (Figs 23.21A and B): **Cysts are lined by non-stratified or stratified cuboidal to columnar epithelial cells** (similar to lining epithelium of normal fallopian tube). Cilia are present though sometimes only focally. May also show:
 - **Papillae with a fibrovascular core**: They are covered by a single layer of epithelium similar to that of the cyst lining.
 - **Psammoma bodies**: They are **dystrophic calcified tumor cells**.
- **Borderline tumors**: They are non-invasive tumors with greater epithelial proliferation and cytological atypia than benign but less than low-grade serous carcinoma.
 - **Stratification of the epithelium (multilayering)** The epithelial proliferation may produce a delicate, papillae pattern termed as “micropapillary carcinoma.” This may be the precursor to low-grade serous carcinoma.
 - **Budding or cellular tufting**: These are tiny, irregular tightly packed stroma-free clusters of tumor cells which gets detached and float into the lumen of the cyst.
 - **Mild nuclear atypia**.
 - **Increased mitotic activity**.
 - **Absence of stromal invasion**.
 - May also show dense and closely packed **complex papillae** and **psammoma bodies**.

- **Malignant: Serous carcinoma based on molecular changes is classified a low-grade or high-grade serous carcinomas [Table 23.2. (50–60%)]**

- **Low-grade serous carcinomas**: It shows variety of architectural patterns. These include single cells and small nets of irregular shape infiltrating the stroma, and micropapillae (rarely macropapillae). The tumor cells are more uniform with mild to moderate nuclear atypia and limited nuclear pleomorphism.

High-grade serous carcinomas: They are distinguished from low-grade carcinoma by the following features:

- More complex growth patterns with solid masses of tumor cells with slit-like spaces.
- Widespread invasion or frank effacement of the underlying stroma (Fig 23.21C).
- Tumor cells show marked **nuclear atypia, pleomorphism, hyperchromatic nuclei, atypical mitotic figures, and large bizarre form or multinucleation**. These cells in invasive high-grade serous carcinoma may become undifferentiated and the serous features may not be evident on microscopic examination.
- **Psammoma bodies**: These are concentric calcifications observed in serous tumors. However, they are not specific for neoplasia.

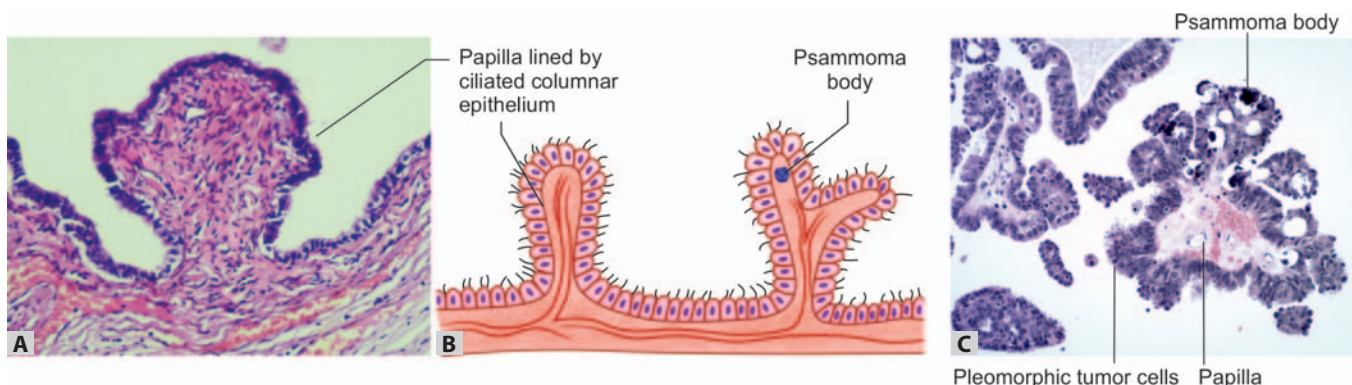
Serous tubal intraepithelial carcinomas: The tumor cells are appear similar to high-grade serous carcinomas but does not show invasion.

Psammoma bodies:

- Laminated calcified concretions
- Seen in serous tumors (both benign and malignant)
- Not specific for ovarian tumors.

TABLE 23.2: Molecular basis of classification of serous carcinoma

Type of carcinoma	Low-grade (well-differentiated)	High-grade (moderately to poorly differentiated)
Carcinoma arises	In serous borderline tumors	In the fallopian tube fimbriae or from serous inclusion cysts within the ovary
Molecular changes: Mutations		
<i>KRAS</i> or <i>BRAF</i> oncogenes (signal transduction)	Present (50-60%)	Absent
<i>TP53</i> gene mutations (tumor suppressor gene)	Rare	High (almost all)
<i>BRCA1/BRCA2</i> mutations (tumor suppressor gene)	Not seen	Seen (50%)



Figs 23.21A to C: Microscopic appearance of papillary serous cystadenoma of ovary showing cyst wall and papilla lined by single layer of cuboidal epithelium. (A) Hematoxylin and eosin (H and E); (B) (Diagrammatic); (C) Papillary serous adenocarcinoma of ovary showing papillae lined by multilayered columnar epithelium and one core of papilla with psammoma body (H&E)

Spread of Ovarian Serous Carcinoma

Local spread: Both low- and high-grade carcinomas may spread to the peritoneal surfaces and omentum and are commonly associated with ascites. Their spread beyond ovary determines the stage of the disease.

Sister Joseph's nodule: Umbilical metastasis from carcinoma (e.g. gastric carcinoma serous carcinoma of ovary).

- **Umbilical metastasis** ("sister Joseph's nodule") (refer pages 490–491).
- Contralateral ovary.
- Abdominal viscera (bowel, liver, spleen)

Lymphatic spread: Para-aortic and pelvic lymph nodes.

Hematogenous spread: Liver and lung.

Mucinous Tumors

Incidence

- Mucinous tumors are **less common than serous tumors**, constitute ~ **30% of all ovarian neoplasms**.
- About **80% are benign or borderline** and about **15% are malignant**.
- Primary ovarian mucinous carcinomas are uncommon and account for less than 5% of all ovarian cancers.
- **Age group:** It is seen mainly during **middle adult life** and are rare before puberty and after menopause.

Molecular Pathogenesis

- **Risk factor:** Smoking.
- **Mutations in KRAS (oncogene):** This may occur early and may be **observed in benign, borderline** and in **primary ovarian mucinous carcinomas**.

MORPHOLOGY

- **General features:** Mucinous tumors **differ from the serous type** in following features.
 - **Larger size.**
 - **Multiloculated** and show hundreds to thousands of small cysts filled with sticky, gelatinous mucinous fluid rich in glycoproteins.
 - **Less bilateral:** Only 5% of mucinous cystadenomas and mucinous cystadenocarcinomas are bilateral.
 - **Surface involvement:** Rare.

Gross

- **Benign-mucinous cystadenoma** (Fig. 23.22):
 - **Multilocular**, thin-walled cysts with smooth external surface.
 - **Cyst content:** Sticky semi-solid mucinous material.
- **Borderline:** Similar to mucinous cystadenoma.

• Carcinoma:

- Similar to mucinous cystadenoma with additional **solid regions**.
- lining of tall, columnar epithelial cells with apical mucin that lack cilia.
- Solid areas show **necrosis and hemorrhage**.

Microscopy

- **Benign mucinous cystadenoma** (Fig. 23.23) are composed of multiple cysts and glands. These are lined by simple non-stratified tall, non-ciliated, columnar mucinous cells with apical mucin and basally situated nuclei. This epithelium resembling gastric foveolar-type or intestinal epithelium. Uncommonly, epithelium may show endocervical type mucinous differentiation.
- **Borderline:** Same criteria as borderline serous tumors, although papillary **projections are less conspicuous**. The cysts are lined by gastrointestinal type of epithelium. The characteristic features are:
 - Stratification of the epithelium/multilayering.
 - Budding or cellular tufting and/or papillary intraglandular
 - growth (appear similar to tubular adenomas or villous adenomas of the intestine).
 - Mild nuclear atypia.
 - Increased mitotic activity.
 - Absence of stromal invasion.
- **Mucinous carcinomas:** It may range from well to poorly differentiated carcinomas. Common features are:
 - **Epithelial cell stratification** and **atypia:** The atypical epithelium is more than four cells in thickness.
 - **Loss of gland architecture.**
 - **Necrosis.**
 - Confluent glandular growth which is a form of "expansile" invasion.
 - Infiltration of the serosa is common.

Spread of Mucinous Carcinoma

Ovarian cancer: Risk increases as age advances.

- **Peritoneal implant** and local invasion into neighboring structures like bowel, abdominal wall, and bladder.
- **Pseudomyxoma peritonei:** It is characterized by:
 - **Extensive mucinous ascites.**
 - **Cystic epithelial implants on the peritoneal surfaces.**

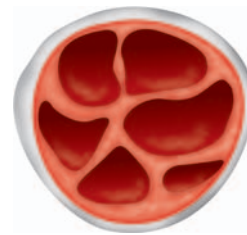
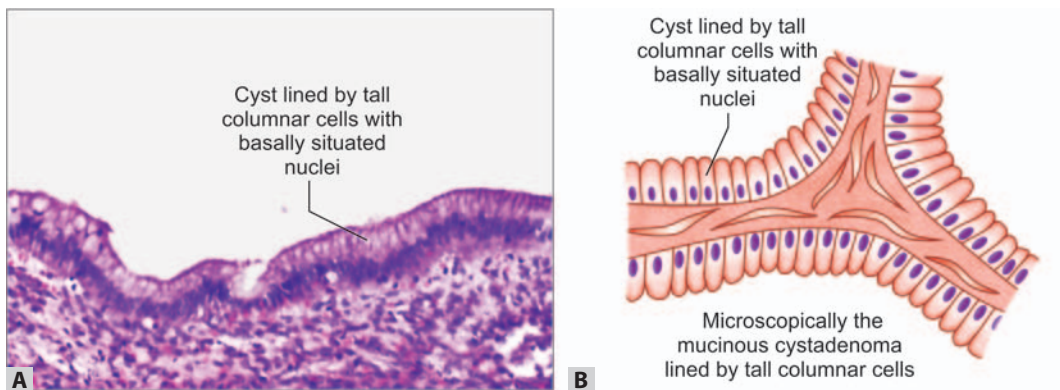


Fig. 23.22: Diagrammatic appearance of mucinous cystadenoma of ovary



Figs 23.23A and B: Microscopic appearance of mucinous cystadenoma of ovary. (A) Photomicrograph; (B) Diagrammatic representation shows cyst lined by uniform, gastrointestinal type of mucinous, columnar cells having basally situated nuclei and apical mucin

- Peritoneal adhesions can cause intestinal obstruction.
 - ◆ It can also develop due to mucinous tumor of the appendix with secondary ovarian and peritoneal spread.
- **Metastases to distant organs are infrequent.**

Most cases of pseudomyxoma peritonei result from spread of a mucinous tumor located in the appendix (mucocele).

Endometrioid Tumors

- Endometrioid tumors show **tubular glands resembling benign or malignant endometrium**.
- **Benign** endometrioid tumors (endometrioid adenofibromas) and **borderline** endometrioid tumors are rare. But, **endometrioid carcinomas** account for approximately 20% of all ovarian cancers.

Pathogenesis

- About 15–20% of cases with **endometrioid carcinoma** coexist with **endometriosis**.
- About 15–30% are **accompanied by carcinoma of the endometrium**.

Molecular Changes Similar to Endometrial Endometrioid Carcinoma

- Frequent mutations in PTEN, PIK3CA, ARID1A, and KRAS), mismatch DNA repair genes and CTNNB1 (β -catenin). TP53 mutations are common in poorly differentiated tumors.

MORPHOLOGY

Gross

Endometrioid carcinomas:

- Size varies from 2–30 cm.

- **Most are solid** and exhibit necrotic areas, but some may show combination of solid and cystic areas.

Microscopy

- Show **glandular patterns resembling endometrial origin**. They are graded similar to endometrial adenocarcinomas (refer pages 671).

Brenner Tumor

Q. Write short note on Brenner tumor of ovary.

Brenner tumor consists of nests of bland transitional-type of cells which resemble urothelial cells within a fibromatous stroma. Brenner tumors are **uncommon** tumors and most of them are benign. Borderline (atypical proliferative Brenner tumor) and malignant Brenner tumors have been rarely reported.

MORPHOLOGY

Gross

- May be **predominantly solid or rarely may be cystic**.
- Usually **unilateral** (approximately 90%).
- Vary in size from small lesions less than 1 cm in diameter to massive tumors up to 20–30 cm.

Microscopy (Fig. 23.24)

- Brenner tumor shows **nests of bland epithelial cells resembling the epithelium of the urinary tract (transitional-type) separated by fibrous stroma**, resembling that of the normal ovary.
- The nuclei of epithelial cells show **nuclear grooves resembling coffee-bean**.

Nuclear grooves resembling coffee-bean:

1. Brenner tumor
2. Granulosa cell tumor.

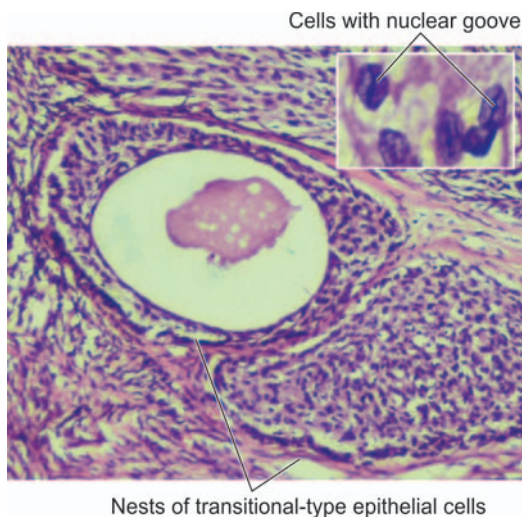


Fig. 23.24: Brenner tumor. Microscopically showing nests of transitional-type cells separated by fibrous stroma. Insert shows tumor cells with nuclear grooves resembling coffee-bean

Clinical Presentation of Surface Epithelial Tumors

- Benign tumors are most common and may be **asymptomatic**.
- May be unilateral or bilateral.
- **Most tumors are nonfunctional** and produce only mild symptoms. The most common symptoms are:
 - **Lower abdominal pain and distention/enlargement.**
 - **Vaginal bleeding.**
 - **Due to compression** produced by the tumor, for example:
 - ◆ Urinary frequency, dysuria.
 - ◆ Gastrointestinal tract symptoms.
- Most **malignant ovarian tumors** are detected when these spread outside the ovary. Symptoms include:
 - **Progressive weakness, weight loss, and cachexia.**
 - **Massive ascites** due to cancer invasion is common and **ascitic fluid may show exfoliated tumor cells.**
 - **Metastasis:**
 - ◆ Regional nodes are often involved.
 - ◆ Hematogenous metastasis to liver, lungs, gastrointestinal tract, and elsewhere.
 - ◆ Across the midline to the opposite ovary.

Biochemical Markers of Surface Epithelial Tumors

- **CA-125:** It is a high-molecular-weight glycoprotein **present in the serum of about 80% of patients with serous and endometrioid carcinomas.** It is **not a reliable mark-**

er because it can be elevated with nonspecific irritation of the peritoneum (e.g. endometriosis, inflammation).

- **Osteopontin:** It is expressed at **higher levels** in ovarian cancer patients.

CA-125: Present in the serum of about 80% of patients with serous and endometrioid carcinomas.

GERM CELL TUMORS

Q. Classify ovarian germ cell tumors.

- Germ cell tumors constitute about **20–25% of all ovarian tumors**.
- **In adults:** Most germ cell tumors are **benign** (mature cystic teratoma, dermoid cyst).
- **In children and young adults:** **Malignant** tumors are the most common.
- They are similar to germ cell tumors in the testis.

Teratomas

Q. Write short note on teratoma of ovary.

Germ cell tumors of ovary: Teratoma is the most common benign germ cell tumor.

Origin

- **All benign ovarian teratomas** have a **karyotype of 46, XX.**
- Teratomas **arise from an ovum after the first meiotic division.**
- They **originate from totipotent cells.**

Totipotent cells: Have the capacity to differentiate into any of the cell types found in the adult body.

- Teratoma contains **mature or immature cells or tissues representative of more than one germ cell layer** (at least two) and sometimes all three embryonic layers. These **cells or tissues are arranged in a helter-skelter fashion** (disorganized).

Sites

- **Gonadal:** Ovary and testis.
- **Extragenital:** Rare and arise from **midline embryonic rests**, e.g. mediastinum, retroperitoneum.

Teratoma:

1. Gonadal
 - Ovary
 - Testis
2. Extragenital.

Classification

1. **Mature (benign) teratoma:** It consists of all **well-differentiated component** parts derived from two or three germ layers (ectoderm, mesoderm and endoderm). Depending on gross feature, they are further categorized as:
 - Cystic (mature cystic teratoma)
 - Solid (mature solid teratoma).
2. **Immature (malignant) teratoma:** It consists of **less well-differentiated or immature elements**.
3. **Monodermal or highly specialized presently called as "monodermal teratoma and somatic-type tumors arising from a dermoid cyst."**

Teratoma:

1. Mature
 - Cystic
 - Solid
2. Immature
3. Monodermal.

However, presently WHO (2014) classifies teratomas as mature, immature teratoma and "monodermal teratoma and somatic-type tumors arising from a dermoid cyst."

Mature (Benign) Teratomas

Q. Write short note on dermoid cyst/benign cystic teratoma of ovary.

- **Most benign ovarian teratomas are cystic** and are better known as **dermoid cysts** (mature cystic teratoma).
- **Age group:** Usually detected in **young women** during the active reproductive years with a peak incidence in the **third decade**.
- Clinically they may be discovered incidentally. The karyotype of benign ovarian teratomas is 46,XX.

MORPHOLOGY

Gross (Fig. 23.25A): Benign teratomas are **bilateral** in 10–15% of cases.

Benign teratomas: Bilateral in 10–15% of cases.

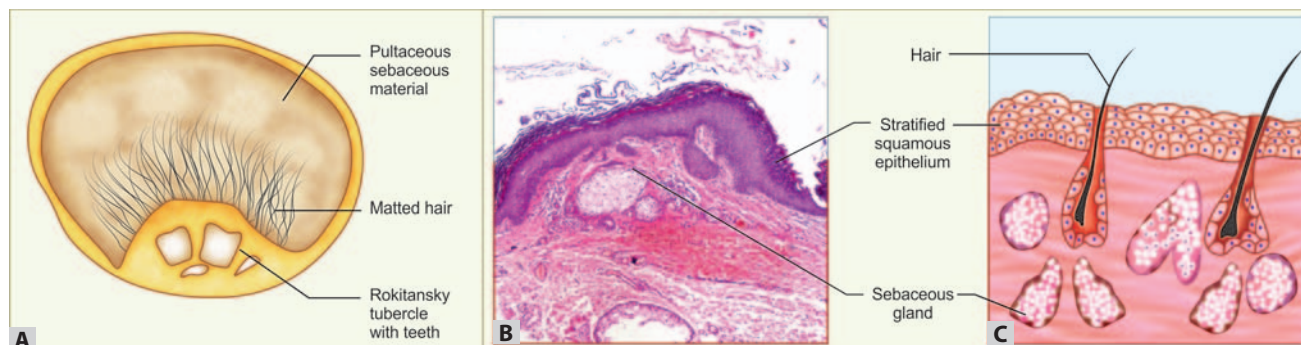
- **Appearance:** It is usually **unilocular, thick-walled cystic tumor** with a smooth, shiny outer surface.
- **Cut section:**
 - Cyst contains yellow or gray, buttery or cheesy **sebaceous material** with variable amount of **hair**.
 - Cyst is **lined** by an opaque, gray-white, **wrinkled epidermis**.
 - **Tooth** and areas of calcification are common.
 - **Rokitansky nodules/protuberance:** These are one or more **foci of rounded nodular structure/s, covered with hair protruding into the lumen** of the cyst. These are also known as the **mammillary body (tubercle)** and **dermoid nipple**. It shows the greatest variety of tissue types of all three germ cell layers and teeth tend to be located at this site.

Microscopy (Figs 23.25B and C)

- These tumors **mainly show differentiation along ectodermal line**. The **cyst wall consists of skin** (stratified squamous epithelium) **with skin appendages** (sebaceous glands, hair shafts, and other skin adnexal structures).
- In most cases, **structures from all three germ layers can be identified**, which includes:
 - Ectoderm (e.g. skin, neural tissue, glia)
 - Mesoderm (e.g. smooth muscle, cartilage, bone, fat)
 - Endoderm (e.g. respiratory tract epithelium, gut, thyroid)

Malignancy in Mature Cystic Teratomas

- One of the mature cellular elements may undergo malignant change in about 1% of the dermoids. They tend to occur in older women and include **squamous cell carcinoma, thyroid carcinoma, melanoma, basal cell carcinoma, and carcinoid tumor**.



Figs 23.25A to C: (A) Opened mature cystic teratoma (dermoid cyst) of the ovary; (B) Photomicrograph; (C) Diagrammatic appearance of dermoid cyst of ovary

Solid Teratoma

- Rarely mature (benign) teratoma can be solid with mature tissues derived from two or three germ layers. On gross examination, these tumors are difficult to differentiate from the malignant, immature teratomas.

Immature Malignant Teratomas

- Immature teratomas are rare tumors **composed of variable amounts of tissues, which resemble embryonal and immature fetal tissue.**
- **Age:** Mostly found in **prepubertal adolescents and young women.** The mean age is 18 years.

Immature teratoma:

- Predominantly solid
- Composed of immature embryonal or fetal tissue admixed with mature tissue.

MORPHOLOGY

Gross: Tumors are **bulky with smooth external surface.**

- **Cut section:**
 - **Predominantly solid** and have lobulated and **variegated appearance**, showing heterogeneous mixture of various tissues.
 - Solid areas may show **grossly recognizable immature bone and cartilage, hair, sebaceous material**, and calcification.
 - Show **areas of necrosis and hemorrhage.**

Microscopy

- Varying amounts of **immature tissues** mixed with some mature tissues derived from the two or three germ layers. The immature elements include **immature neuroepithelium (neuroepithelial rosettes and immature glia)**, cartilage, bone, muscle, and others.

Grading: It is based on the amount of immature neuroepithelium.

Monodermal Teratomas and Somatic-type Tumors Arising from a Dermoid Cyst

Q. Write short note on struma ovarii.

- Specialized teratomas are **rare tumors composed entirely of one tissue type.** The most common among these rare are struma ovarii and carcinoid.

Struma Ovarii

Monodermal/specialized teratoma: Rare type of teratoma composed entirely of one tissue type.

- It is a **cystic lesion** composed entirely or predominantly of **mature thyroid tissue** (Fig. 23.26). These thyroidal neoplasms may hyperfunction and may lead to hyperthyroidism.

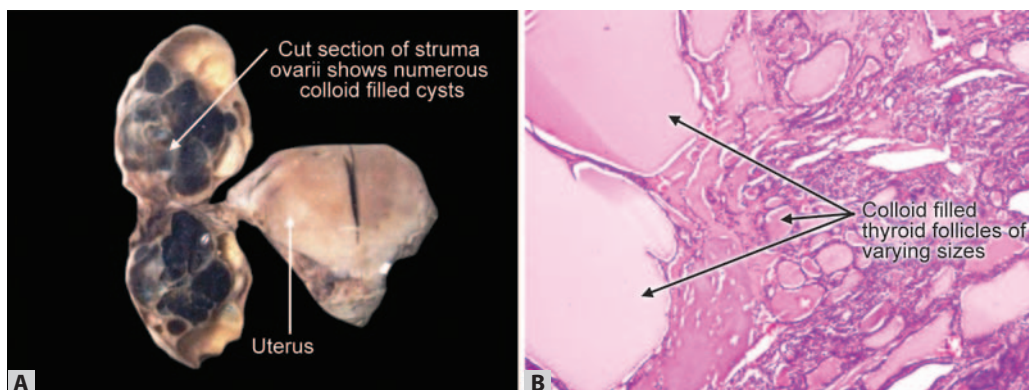
Ovarian Carcinoid

- Probably arise from intestinal epithelium in a teratoma. They may be functional (producing 5-hydroxytryptamine) and result in carcinoid syndrome.
- **Strumal carcinoid:** It is a combination of struma ovarii and carcinoid in the same ovary.

Dysgerminoma

Germ cell tumors of ovary: Dysgerminoma is the most common malignant tumor.

- Dysgerminoma is the **ovarian counterpart of the seminoma of the testis.**
- Constitutes ~2% of all ovarian cancers and ~50% of malignant germ cell tumors.
- **Age group:** ~75% occur in the **second and third decades.**
- **Predisposing factors:** **Gonadal dysgenesis** (including pseudohermaphroditism) is one of the risk factors.



Figs 23.26A and B: (A) Cut section of struma ovarii involving the ovary; (B) Struma ovarii composed of thyroid follicles containing variable amount of colloid

- **Functional characteristics:** Most do not show endocrine function.
- **Molecular changes:**
 - Like seminomas, dysgerminomas **express transcription factors, namely Oct3, Oct4, and Nanog.** These are responsible for pluripotency.
 - They also **express the receptor tyrosine kinase KIT.**

Dysgerminoma: Ovarian counterpart of seminoma of testis.

MORPHOLOGY

Q. Write short note on morphology of dysgerminoma.

Gross

- Usually **unilateral** (80–90%)
- **Solid, firm, round to oval, encapsulated** tumors.
- **External surface:** Smooth, nodular, convoluted/bosselated.
- **Size:** Varies with a mean diameter 15 cm.
- **Cut section** (Fig. 23.27A): **Soft, fleshy** and uniformly **yellow-white to gray-pink** (cream colored).

Microscopy (Figs 23.27B and C)

Dysgerminoma:

- Sheets of tumor cells separated by scanty fibrous stroma
- Tumor cells are monotonous
- Stroma infiltrated by mature lymphocytes.
- **Pattern:** The tumor cells are arranged in **sheets, groups, cords or nests** separated by **scant fibrous stroma**.
- **Tumor cells:** Resemble **primordial germ cells** with following characteristics:
 - **Cells:** Individual tumor cells are **monotonous (uniform).**
 - **Cytoplasm:** It is **abundant clear to finely granular glycogen-filled** and sometimes fine droplets of fat. The **cell membrane is well-defined.**

- **Nuclei:** They are **large, regular** and **centrally placed** with **fine reticular chromatin** and one or more **prominent nucleoli.**
- **Mitotic figures** are usually **numerous.**
- **Stroma:** Similar to seminoma, the fibrous stroma is **infiltrated with mature lymphocytes** (most are of T-cell type) and occasional granulomas.

Spread

- **Local spread:** It can spread into peritoneal cavity and is associated with a decreased survival rate.
- **Lymphatic spread:** Through lymphatics, it spreads commonly to the contralateral ovary and retroperitoneal nodes.
- **Blood spread:** Lungs.

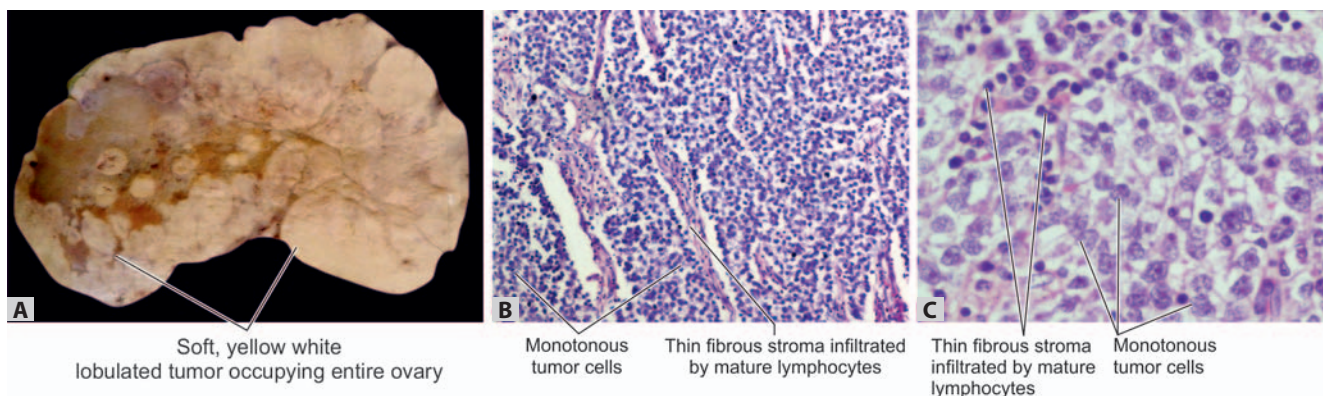
Prognosis: All dysgerminomas are **malignant**. Dysgerminoma is treated surgically, and 5-year survival for patients with stage I is almost 100%. The tumor is **highly radiosensitive** and also responsive to chemotherapy.

Dysgerminoma: Though suffix is 'oma', it is malignant germ cell tumor.

Endodermal Sinus (Yolk Sac) Tumor

- Endodermal sinus tumor is the **second most common malignant tumor of germ cell tumor.**
- **Origin:** From **differentiation of malignant germ cells along the extraembryonic yolk sac lineage.**
- Tumor is **rich in α -fetoprotein and α_1 -antitrypsin.**
- **Age:** **Children or young women.**

Endodermal sinus tumor: Malignant aggressive, rapidly growing germ cell tumor. Can also occur in testis.



Figs 23.27A to C: Dysgerminoma (A) Gross showing a solid tumor, cut section appears fleshy and cream colored; (B) Low magnification and (C) High magnification) composed of polyhedral tumor cells with clear cytoplasm reticular nuclei and adjacent scanty fibrous stroma infiltrated by lymphocytes

MORPHOLOGY

- **Gross:** Usually unilateral and solid.

Microscopy

- **Schiller-Duval body:** It is the **characteristic feature**. This is a **glomerulus-like structure composed of a central blood vessel enveloped by germ cells within a space lined by germ cells** (Fig. 23.28).
- **Hyaline droplets:** All show **prominent intracellular and extracellular PAS positive hyaline droplets**. Some of these may stain for α -fetoprotein by immunoperoxidase techniques.

Endodermal sinus tumor:

1. Schiller-Duval body
2. PAS positive hyaline droplets.

Schiller-Duval body: Glomerulus-like structure composed of a central blood vessel enveloped by germ cells within a space lined by germ cells.

Tumor Marker

- α -fetoprotein and α_1 -antitrypsin in tumor cells by immunohistochemistry
- α -fetoprotein in the serum

Endodermal sinus tumor:

1. α -fetoprotein
2. α_1 -antitrypsin.

Prognosis: Aggressive and rapidly growing tumor.

Nongestational Choriocarcinoma

Choriocarcinoma:

1. Gestational
2. Nongestational.

Choriocarcinoma in females may be:

- **Gestational:** More common of **placental origin**.
- **Nongestational:** Germ cell origin can be confirmed only in the prepubertal girl. This is because after puberty the origin from an ovarian, ectopic pregnancy cannot be ruled out.

Pure choriocarcinomas of ovary are extremely rare and most coexist with other germ cell tumors.

Choriocarcinoma: Consists of cytotrophoblast and syncytiotrophoblast without any villi.

MORPHOLOGY

- **Gross:** Solid with large areas of hemorrhage
- **Microscopy:** Similar to gestational choriocarcinoma (refer page 690) and consists of cytotrophoblast and syncytiotrophoblast without any villi.
- **Behavior:** Aggressive tumors and generally metastasize through the bloodstream to the lungs, liver, bone, and other viscera at the time of diagnosis.

Choriocarcinoma: Secretes hCG.

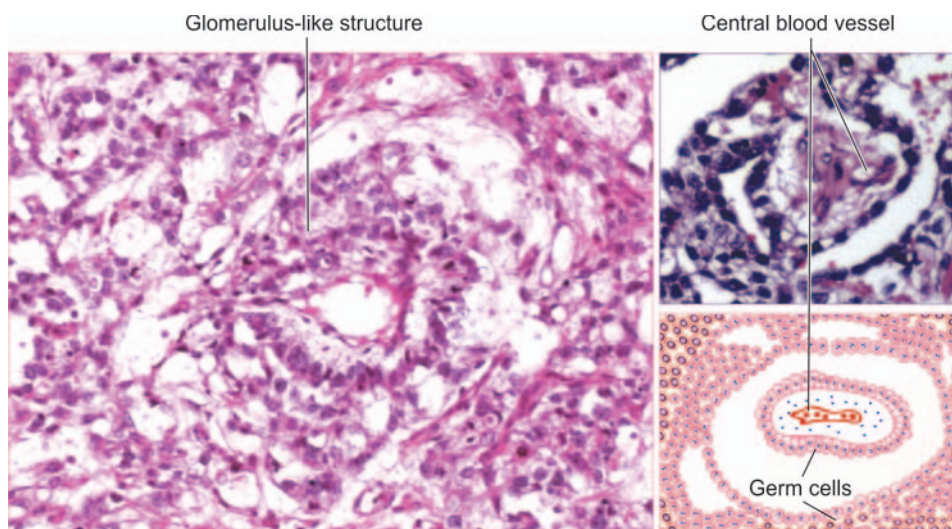


Fig. 23.28: Endodermal sinus (yolk-sac) tumor showing characteristic glomerulus-like structure known as Schiller-Duval body. It is composed of a central blood vessel enveloped by germ cells within a space lined by germ cells. Inset (upper right-H&E) and lower right (diagrammatic) showing Schiller-Duval bodies

- **Tumor marker:** These tumors secrete high levels of chorionic gonadotropins (hCG), which help in diagnosis or detecting recurrences.
- **Prognosis:** In contrast to gestational, these nongestational types generally do not respond to chemotherapy and are fatal.

Other Germ Cell Tumors

- **Embryonal carcinoma:** Highly malignant tumor of primitive embryonal elements, similar to that of testes.
- **Mixed germ cell tumors:** They show various combinations of dysgerminoma, teratoma, endodermal sinus tumor and choriocarcinoma.

SEX CORD-STROMAL TUMORS

Sex cord-stromal tumors: Produce hormones and most are benign.

- Ovarian stroma is derived from the sex cords of the embryonic gonad. So, this group of tumors is known as sex cord-stromal tumors.
- **Hormonal activity:** Some of these cells normally secrete estrogens (granulosa and theca cells) or androgens (Leydig cells). The corresponding tumors may be either feminizing (granulosa-theca cell tumors) or masculinizing (Leydig cell tumors).

Sex cord-stromal tumors: Produce estrogen or androgens.

Granulosa-Theca Cell Tumors

Q. Write short note on granulosa cell tumor of ovary.

- Granulosa cell tumor of the ovary is associated with estrogen secretion.
- These neoplasms may be composed almost entirely of granulosa cells or a varying mixture of granulosa and theca cells.
- **Incidence:** Account for about 5% of all ovarian tumors.
- **Age group:** About two-thirds occur in postmenopausal women.

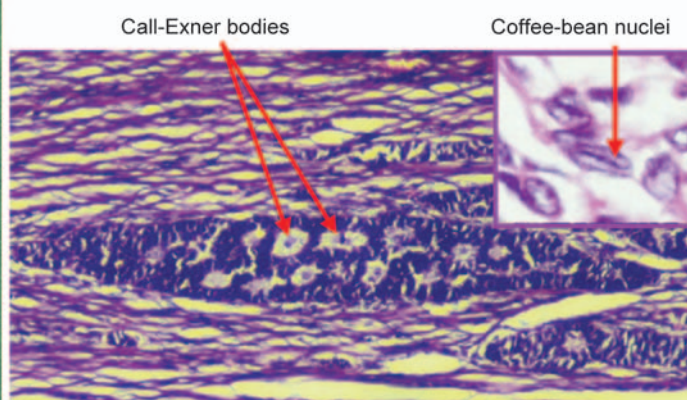
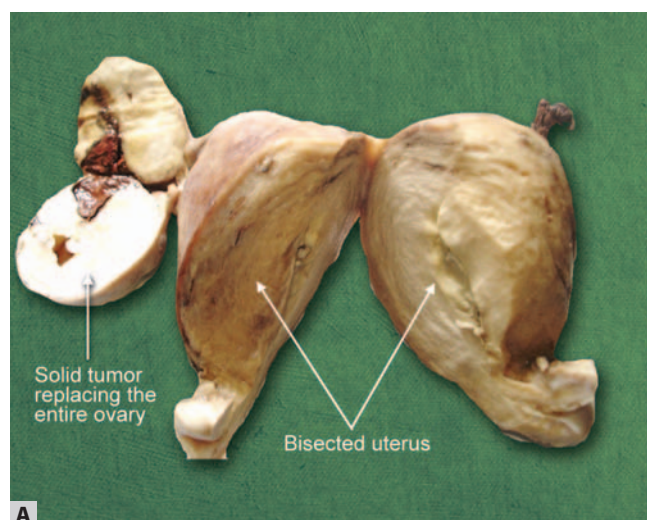
MORPHOLOGY

Gross (Fig. 23.29A)

- Usually **unilateral**.
- **Size varies** from microscopic foci to large, solid, and cystic encapsulated masses.
- **Cut surface:** **Hormonally active tumors** have a **yellow color**, due to intracellular lipids (lipid-laden luteinized granulosa cells).

Microscopy

- **Granulosa cell component (Fig. 23.29B):** Tumor cells are **small, cuboidal, polygonal cells to spindle-shaped** and commonly have a **cleaved, elongated nucleus (coffee bean appearance—Fig. 23.29B inset)**.
 - **Growth patterns**
 - ♦ **Diffuse** (sarcomatoid) sheets
 - ♦ **Insular** (islands of cells)
 - ♦ **Trabecular** (anastomotic bands of granulosa cells)



Figs 23.29A and B: Granulosa cell tumor of ovary. (A) Gross appearance shows solid tumor; (B) Photomicrograph composed of tumor cells are arranged in sheets punctuated by small follicle-like structures (Call-Exner bodies). Inset of B shows coffee-bean nucleus of tumor cells

- **Call-Exner bodies** (Fig. 23.29B): These are **small, distinctive, gland-like** (follicular structures/degenerative) **space filled with an acidophilic material** and appear like an immature follicles. These characteristic structures when present are **very useful for diagnosis**.
- **Thecoma component:** It consists of **clusters or sheets of cuboidal to polygonal cells**.

Granulosa cell tumor:

- Call-Exner bodies
- Cells with coffee bean nucleus.

Clinical Features

The presenting **clinical features may be due to** secretion of hormones mainly **estrogen** and occasionally androgen. They **vary depending on the age** of the patient.

- **Prepubertal girls** (juvenile granulosa cell tumors): It may produce **precocious sexual development**.
- **Adult women:** They may cause **endometrial hyperplasia, cystic disease of the breast, and endometrial carcinoma**.
- **Occasionally**, they secrete **androgens** and results in **masculinizing** of the patient.

Behavior: All granulosa cell tumors are considered as **potentially malignant** because it may spread locally as well as metastasize. Tumors composed predominantly of theca cells tend to be benign.

Granulosa cell tumor:

- Potentially malignant
- Secrete estrogens.

Biochemical markers: **Inhibin** is secreted by granulosa cells and **elevated tissue and serum** levels of which are useful for identifying granulosa tumor and monitoring treated patients. Mutations of the **FOXL2** gene is observed in 97% of adult granulosa cell tumors.

Granulosa cell tumor: Marker is inhibin.

Fibromas, Thecomas, and Fibrothecomas

Q. Write short note on Meigs syndrome.

Tumors arising from ovarian stroma that are composed of:

- **Only fibroblasts** are called **fibromas** and are hormonally inactive.
- **Plump spindle cells with lipid droplets** are **thecomas**. Pure thecomas are rare, but may be hormonally active.
- **Mixture** of the above two types of cells are known as **fibrothecomas**.
- Majority of fibromas, thecomas, and fibrothecomas are **benign**.

MORPHOLOGY

Fibroma

- **Unilateral** in about 90% of cases.
- Usually **solid, spherical or slightly lobulated, encapsulated, hard, gray-white** masses covered by glistening, intact ovarian serosa.
- **Microscopy:** Consist of **well-differentiated fibroblasts separated by scant collagenous connective tissue**. Focal areas of thecal differentiation may be found.

Clinical Features

- Usually present as a **pelvic mass** sometimes accompanied by pain.
- **Associated features:**
 - **Meigs syndrome:** It is the combination of **ovarian fibroma, hydrothorax, and ascites**. The mechanism is not known.
 - **Ascites** found in about 40% of cases when **tumors are larger than 6 cm** in diameter.
 - **Uncommonly, hydrothorax** usually only of the **right side**.
 - Fibroma may be found in association with basal cell nevus syndrome.

Meigs syndrome:

1. Ovarian fibroma
2. Hydrothorax usually on right side
3. Ascites.

METASTATIC TUMORS

Constitute about 3% of ovarian tumors.

Source of Primary Tumor

- **From genital tumors:** Most common metastatic tumors are derived from tumors of the **uterus, fallopian tube, and contralateral ovary**.
- **Extragenital tumors:** Most common extra-Müllerian tumors metastatic to the ovary are from **carcinomas of the breast and gastrointestinal tract** (including colon, stomach) biliary tract, and pancreas.

Gross features, which point to metastatic carcinoma are:

- **Bilateral** ovarian involvement.
- **Multinodularity**.

Metastatic tumors of ovary: Most common source of metastatic from genital tumors originate from uterus, fallopian tube, and contralateral ovary.

Metastatic tumors of ovary: Most common source of metastatic from extragenital tumors originate carcinoma of breast and GI tract.

Krukenberg Tumors

Q. Write short note on Krukenberg tumor.

- First described by Krukenberg in 1896.
- **Age:** Usually found between **30 and 60 years**.
- **Sources of primary:**
 - **Stomach** is the most common primary site (in 75% of cases).
 - **Large intestine.**
 - **Breast.**

MORPHOLOGY

- **Gross:**
 - Most are **bilateral**.
 - **Moderately enlarged** and **shape of the ovary is maintained**.
 - **Capsule is intact and smooth.**
 - Cut section shows a **solid tumor with variegated appearance**.
- **Microscopy:** It shows **nest of mucin-producing, signet-ring cancer cells within a cellular stroma** of the ovary.

Krukenberg tumor: Microscopy shows nests of mucin-producing, signet-ring cancer cells within a cellular stroma of the ovary.

Krukenberg tumor:

- Metastatic tumor of ovary
- Most common primary is from stomach.

GESTATIONAL DISORDERS

GESTATIONAL TROPHOBLASTIC DISEASE

Gestational trophoblastic disease consists of tumors and tumor-like lesions characterized by proliferation of placental tissue (villous or trophoblastic).

Gestational trophoblastic disease:

- Tumors and tumor-like lesions
- Proliferation of placental tissue (villous or trophoblastic).

Classification (Box 23.5)

WHO (2014) classification of gestational trophoblastic disease is enlisted in (Box 23.5).

BOX 23.5: WHO (2014) Classification of gestational trophoblastic disease (abridged)

Molar pregnancies

- Hydatidiform mole
 - Complete
 - Partial
 - Invasive mole

Neoplasms

- Choriocarcinoma
- Placental-site trophoblastic tumor

Non-neoplastic lesions

- Exaggerated placental site

Hydatidiform Mole

Hydatidiform mole: Benign non-neoplastic proliferative disorder of the placenta.

Definition: Hydatidiform mole is **benign, non-neoplastic, gestational trophoblastic disease of placenta** characterized histologically by **cystic swelling of the chorionic villi**, accompanied by **variable trophoblastic proliferation**. Androgenetic diploidy (diploid paternal-only genome) is the genetic cause in majority of cases.

Significance: Hydatidiform mole is **associated with an increased risk of invasive mole or choriocarcinoma**.

Age: Mostly present in the **fourth or fifth month of pregnancy with vaginal bleeding**. Currently, due to routine ultrasound examination during early pregnancy, moles are detected at earlier gestational ages.

Hydatidiform mole: Increased risk of invasive mole or choriocarcinoma.

Risk Factors

Hydatidiform mole: Complete or partial.

- **Maternal age:** It has two peaks.
 - Risk is higher in girls **younger than 15 years** of age.
 - Risk increases progressively **after 40 years**.
- **Ethnic background** and **obstetric history** also **influence the risk** of developing hydatidiform mole.

Types

Q. Write short note on differences between complete and partial mole.

Depending on cytogenetic and histological features, benign, noninvasive moles are divided into **two types**.

Complete Hydatidiform Mole (Figs 23.30A and B)

Complete hydatidiform mole: Whole placenta is neoplastic without any fetal parts and are diploid.

- Complete mole **results from fertilization of an egg that has lost its chromosomes, either by one or two sperms**. Thus, the **characteristic feature is complete absence of maternal chromosomes** (empty ovum) and the **genetic material is completely paternally derived** (from sperm).
- **Most commonly (~90%)**, complete moles develop from **fertilization of an empty egg/ovum by a one sperm**. The **genetic material/chromosomes of the sperm (23,X not 23,Y) undergoes duplication** a phenomenon called **androgenesis**. Thus, the complete mole formed has a **karyotype 46,XX** diploid pattern, all derived from sperm. **Never 46YY**.
- **Less commonly (~10%)**, complete moles are formed by the **fertilization of an empty ovum by two sperm (dispermy)**. Depending on genetic material of the two sperms (23,X or 23,Y), the complete mole has either **karyotype 46,XX** (two sperms with 23,X) or **46,XY** (one sperm with 23,X and other with 23,Y).

Complete hydatidiform mole: Hydropic avascular villi with trophoblastic proliferation.

Complete mole: Only 2% develop choriocarcinoma.

Partial Hydatidiform Mole (Fig. 23.30C)

Partial mole: Part of placenta neoplastic, fetal parts present and triploid (karyotyping shows 69 chromosomes).

- Partial moles result from **fertilization of a single ovum with two sperms** (23,X or 23,Y). Thus, in these partial moles the **karyotype is triploid** {(e.g. **69,XXY**- ova (23X) + sperm (23X + 23Y) or **69,XYY**- ova (23X) + sperm (23Y + 23Y)} or even occasionally tetraploid (92,XXXXY).
- **Fetal parts are commonly present**.
- In contrast to complete mole, there is **no increased risk for choriocarcinoma**.

MORPHOLOGY

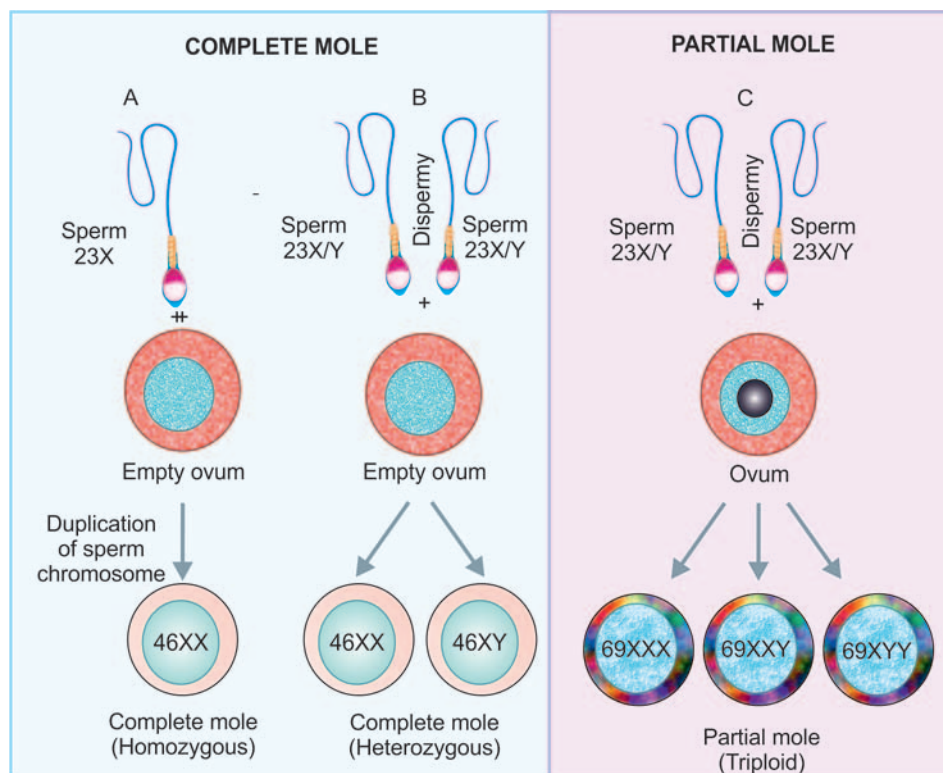
Q. Write short note on morphology of hydatidiform mole.

Gross (Figs 23.31A and B)

Complete hydatidiform mole represents a placenta with grossly swollen, edematous (hydropic) chorionic villi.

- **Hydropic chorionic villi appear as delicate and form a friable thin-walled, translucent, cystic mass which resemble bunches of grapes.**

Fetal parts are frequently seen in partial moles.



Complete mole:

- Diploid
- All chromosomes are paternal
- No embryonic or fetal tissue.

Complete mole: The genetic material/chromosomes of the sperm (23,X not 23,Y) undergoes duplication a phenomenon called androgenesis.

Partial mole:

- Triploid
- Two sets of paternal chromosomes
- Fetal tissue present.

Figs 23.30A to C: Schematic representation of origin of complete and partial hydatidiform moles. (A) More commonly complete moles develop from fertilization of an empty ovum by a single sperm that undergoes duplication of its chromosomes; (B) Less commonly, complete moles may arise from fertilization of an empty ovum by two sperms (dispermy); (C) Partial moles develop from fertilization of single ovum by two sperm and forms partial mole with triploid karyotype

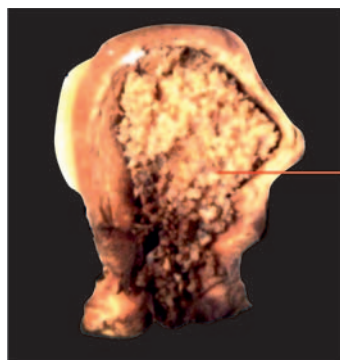
Microscopy of Complete Mole (Fig. 23.32)

Abnormalities involve all or most of the villi.

- **Characteristics of villi**
 - Individual chorionic villi are **edematous, enlarged, scalloped in shape**.
 - The **central areas of the villi are acellular, devoid of mesenchymal cells, and lack adequately developed vessels**.
 - They show **fluid-filled spaces [cavitation (cisterns/cisternae)]**
- **Trophoblast proliferation**
 - An **extensive/diffuse trophoblast proliferation (hyperplasia)**, which involves the **entire circumference of the villi**.
 - Trophoblast consists of **syncytiotrophoblast, cytotrophoblast and intermediate trophoblast**.
 - They may show cellular atypia.
- **Fetal parts are absent.**

Microscopy of Partial Mole

Histological differentiation of complete mole from partial molar is important.



Uterus filled with grape-like vesicles

Fig. 23.31: Complete hydatidiform mole showing marked distention of the uterus by grape-like vesicular chorionic villi

- Partial moles have **two populations of chorionic villi**:
 - **Normal villi**.
 - **Swollen (edematous) villi** due to hydropic swelling and central cavitation.
- The **trophoblastic proliferation is moderate, focal and less marked** than that seen in complete mole.
- Chorionic villi show **blood vessels with fetal (nucleated) erythrocytes**.

Clinical Features

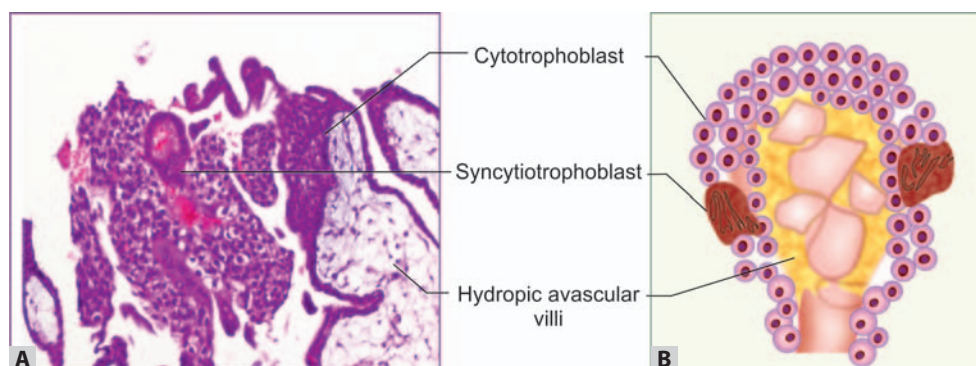
- Both partial and early complete moles present with **spontaneous pregnancy loss**.
- **Human chorionic gonadotropin (β -hCG)**:
 - In **complete moles, β -hCG levels are high** compared to normal pregnancy.
 - Serial hormone determination shows increase in the β -hCG levels faster than for the pregnancy.
- **Monitoring serum concentrations of β -hCG is necessary** to determine the early development of invasive moles or gestational choriocarcinoma.
- Majority of moles are treated by thorough curettage.

Hydatidiform mole: Secretes high levels of β -hCG.

Invasive Mole

Invasive mole:

- Invades or perforates uterine wall
- Shows villi with trophoblastic proliferation.
- **Definition:** It is defined as a **hydatidiform mole, complete or partial that penetrates or even perforates the uterine wall**.
- **Microscopy:** Invasion of the myometrium by **hydropic chorionic villi**, accompanied by **proliferation of both cytotrophoblast and syncytiotrophoblast**.



Figs 23.32A and B: Complete hydatidiform mole showing marked swollen chorionic villi and circumferential trophoblast proliferation

- **Spread:**
 - **Local spread:** It can spread into parametrial tissue and blood vessels.
 - **Blood spread:** Lungs and brain.
- **Clinical features:** Present as **vaginal bleeding** and **irregular uterine enlargement**.
- **Laboratory findings:**
 - **Persistently elevated** serum β -hCG.
 - **Varying degrees of luteinization of the ovaries.**
- **Treatment:** Responds well to **chemotherapy**.
- **Nongestational:**
 - **Very rare**
 - **Develop from germ cells in the ovaries/testis or the mediastinum.**

Choriocarcinoma

Gestational choriocarcinoma: Highly invasive malignant neoplasm of trophoblastic cells which metastasizes widely.

Definition: Gestational choriocarcinoma is a **rapidly invasive malignant neoplasm of trophoblastic cells**, which **metastasizes widely**. It responds well to **chemotherapy**.

Types

Q. Write short note on choriocarcinoma.

Choriocarcinoma may be subdivided into gestational and nongestational.

- **Gestational:** It is preceded by:
 - Hydatidiform moles
 - Previous abortions
 - Normal pregnancies
 - Ectopic pregnancies.

MORPHOLOGY

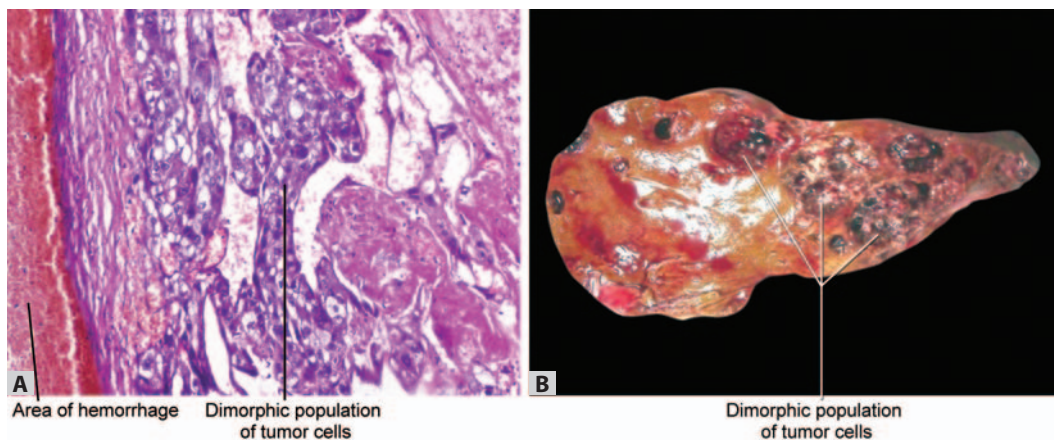
Gross

- **Size:** Choriocarcinoma in the uterus **range from microscopic foci to huge necrotic and hemorrhagic tumors**.
- **Appearance:**
 - **Soft, fleshy, yellow-white tumor**
 - **Secondary changes**
 - ♦ Large pale areas of ischemic necrosis
 - ♦ Extensive areas of hemorrhage
 - ♦ Foci of cystic softening.

Microscopy (Fig. 23.33A)

Choriocarcinoma:

- **Mixed/dimorphic population of syncytiotrophoblasts and cytotrophoblasts**
- **No villi.**
- **Absence of chorionic villi.**
- **Trophoblastic cells:** They consist of a **mixed/dimorphic population of syncytiotrophoblasts and cytotrophoblasts**.
- **Mitosis: Abundant** and sometimes abnormal.
- **Secondary changes:** Due to rapid growth, it can undergo:
 - Hemorrhage
 - Ischemic necrosis
 - Secondary inflammation.
- **Invasion:** They can invade into the underlying **myometrium, blood vessels and lymphatics**.



Figs 23.33A and B: (A) Photomicrograph of choriocarcinoma showing dimorphic population of neoplastic cytotrophoblast and syncytiotrophoblast; (B) Liver with multiple secondaries from choriocarcinoma

Clinical Features

- Uterine choriocarcinoma usually manifests as **irregular vaginal spotting of a bloody, brown fluid**.
- May follow an apparently normal pregnancy, after a miscarriage, or after curettage.

Laboratory findings: β -hCG is elevated more than that found in hydatidiform moles.

Gestational choriocarcinoma: Responsive to chemotherapy and curable.

Spread

Widespread metastases are characteristic of choriocarcinoma. **Frequent sites** include: **Lungs, brain, bone marrow, liver** (Fig. 23.33B), **vagina** and other organs.

Treatment of gestational choriocarcinoma: It consists of:

- Evacuation of the contents of the uterus
- Surgery
- Chemotherapy: Results in nearly 100% remission and a high rate of cures.

Nongestational choriocarcinomas: Resistant to chemotherapy.

Breast Disorders

FEMALE BREAST

Breast is made up of two major components: (1) **terminal duct-lobular unit (TDLU)** and (2) **large duct system** (Fig. 24.1).

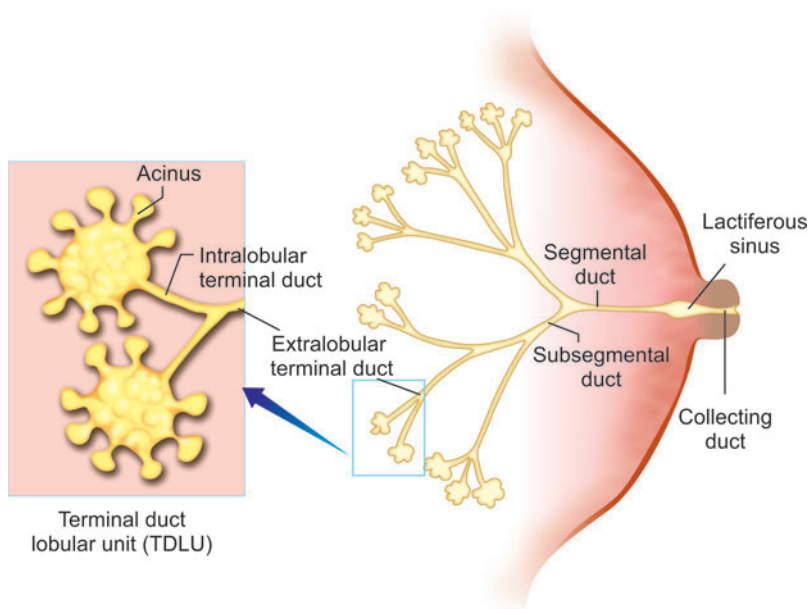
- **TDLU:** It consists of **lobule**, together with its **terminal** (intralobular and extralobular) duct. Each **lobule** consists of a variable number of **terminal ductules/acini** embedded within specialized intralobular stroma and is connected to the intralobular terminal duct.
- **Large duct system:** The intralobular ducts emerge from the lobule as extralobular duct and connects

with the **subsegmental duct**, which in turn leads to a **segmental duct**. This leads to a **collecting (lactiferous or galactophorous) duct**, which opens onto the surface of the nipple. A fusiform dilation is seen beneath the nipple between the collecting and the segmental duct is known as the **lactiferous sinus**.

MICROSCOPY

Normal ducts and lobules: Two specialized cell type lining

1. Inner epithelium with secretory and absorptive function
2. Outer myoepithelial cell.



Biopsy techniques for breast lesions:

1. Fine needle aspiration cytology (FNAC)
2. Tru-cut (core-cut) biopsy
3. Excisional biopsy.

Major components of breast:

1. TDLU
2. Large duct system.

Fig. 24.1: Diagrammatic appearance of breast parenchyma. Terminal duct-lobular unit (TDLU) consists of extralobular terminal duct; intralobular terminal duct and acini

- **Lining of ducts and lobules:** Entire ducts and lobules of the breast is lined by a specialized two-cell-type epithelial lining: The **inner epithelium** with secretory and absorptive functions (often simply called epithelium), and the **outer myoepithelial cells** (refer Fig. 24. 5A).
- **Breast stroma:** It consists of two types namely, **intra-lobular** and **extralobular**.

BENIGN EPITHELIAL LESIONS

Classification: According to the subsequent risk of developing breast carcinoma, the benign epithelial lesions of breast can be divided into three groups:

1. Nonproliferative breast changes
2. Proliferative breast disease
3. Atypical hyperplasia.

Nonproliferative Breast Changes (Fibrocystic Changes)

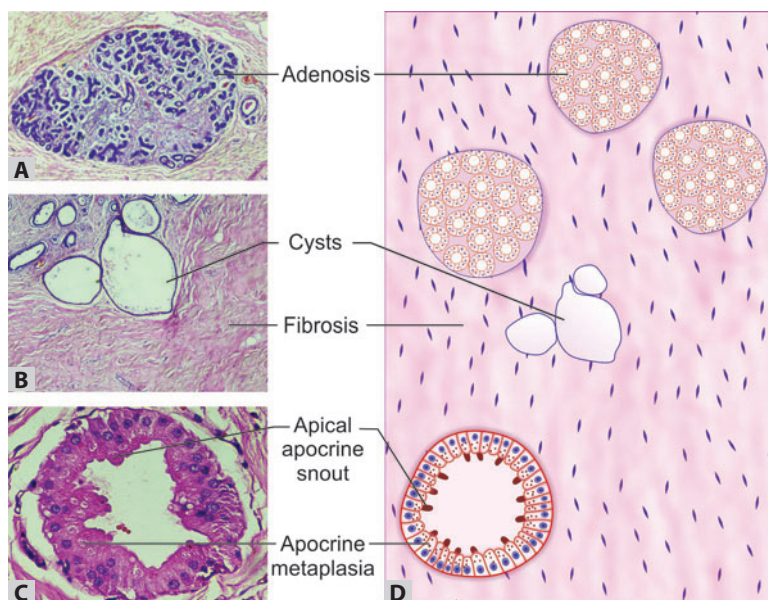
Q. Write short note on fibrocystic disease of breast (fibrocystic changes).

- It is a group that consists of a many **common morphological changes observed in the breast** and is also termed as fibrocystic changes.
- **No increased risk of carcinoma of breast.**

MORPHOLOGY (FIG. 24.2)

Fibrocystic changes **primarily affect the TDLU**. Its characteristic morphological features are:

1. **Cysts:** It can be **microscopic or grossly visible**. Small cysts may coalesce to form larger cysts.
 - **Cysts contain turbid, cloudy yellow and semi-translucent fluid.** Some of these cysts externally appear brown or blue color ('blue dome cysts' of Bloodgood).
 - **Microscopically**, cysts are lined either by a **flattened atrophic epithelium** (especially the larger cysts) or by **metaplastic apocrine cells**.
2. **Apocrine metaplasia:** It is a common change, most often seen in **dilated ducts and cystic structures**.
 - Apocrine cells have an **abundant granular, eosinophilic/acidophilic cytoplasm** and **round nuclei with prominent nucleolus**. The apical portion of the cytoplasm shows the typical '**apocrine snout**'.
3. **Calcification:** It is less common and line the bottom of a rounded cyst and mammographers use the term "milk of calcium" to describe these calcifications.
4. **Fibrosis:** It is usually seen and its degree varies markedly. Chronic inflammation, fibrosis, hyalinization contribute to the firmness of the breast during palpation.
5. **Adenosis:** It is defined as an increase in the number of acini (terminal ductule) per lobule.
 - Acini are often enlarged (blunt-duct adenosis).
 - Acini are lined by columnar cells, which may appear benign or show atypical features. ("**flat epithelial atypia**"). Flat epithelial atypia is probably the earliest recognizable precursor of low-grade breast cancers.



Figs 24.2A to D: Microscopic features of nonproliferative breast change (fibrocystic changes); (A) hematoxylin and eosin (H & E) showing adenosis; (B) (H and E) showing fibrosis and cysts; (C) (H and E) showing cysts with apocrine metaplasia of the lining cells; (D) diagrammatic appearance showing the above features

Fibrocystic changes: Formerly known as fibroystic disease.

Nonproliferative breast changes:

1. Cysts
2. Apocrine metaplasia
3. Calcification
4. Fibrosis
5. Adenosis.

Atypical hyperplasia: Cellular proliferation resembling DCIS or LCIS, but lacking features for a diagnosis of carcinoma in situ.

Milk of calcium: Calcifications in large cysts look as if they are lining the bottom of a rounded cyst on mammography.

Lactational adenoma: They develop as palpable masses in pregnant or lactating women. They show normal-appearing breast tissue with lactational changes. They represent an exaggerated local response to gestational hormones.

Proliferative Breast Disease without Atypia

- Characterized by **proliferation of ductal epithelium and/or stroma without cytologic or architectural features of carcinoma in situ.**

Proliferative breast disease without atypia:

1. Epithelial hyperplasia
2. Sclerosing adenosis
3. Complex sclerosing lesion
4. Papillomas.

MORPHOLOGY

Epithelial hyperplasia: Ducts and lobules lined by more than two layer of cells.

1. **Epithelial hyperplasia:** Normal breast ducts and lobules are lined by two layers of cells: Inner (luminal) epithelial cells and outer myoepithelial cells.
 - **Epithelial hyperplasia** is defined as the **presence of more than two cell layers**. The additional cells consist of both luminal and myoepithelial cell types. These cells fill and distend ducts and lobules. **Irregular lumens can often be seen at the periphery of the cellular masses.**
 - Epithelial hyperplasia may be mild (when made up of three or four epithelial cells in thickness), moderate to florid (when more pronounced), and atypical.
2. **Sclerosing adenosis:** It is a **form of adenosis**. The **number of acini per lobule is increased** and at least double the number found in uninvolved lobules.
 - The normal lobular arrangement is maintained and is **more cellular centrally than peripherally**.
 - The **acini are compressed and distorted in the central portions of the lesion** but dilated at the **periphery**. Myoepithelial cells are usually prominent. On occasion, stromal fibrosis may produce a microscopic **appearance mimicking invasive carcinoma**.
3. **Complex sclerosing lesion:** Its components include sclerosing adenosis, papillomas, and epithelial hyperplasia.
4. **Papillomas:** It consists of **multiple branching central fibrovascular cores lined by luminal and myoepithelial cells**. These **papillae grow within a dilated duct**. Epithelial hyperplasia and apocrine metaplasia are frequently seen.
 - Papillomas are usually **single in large duct** and are found in the lactiferous sinuses of the nipple.

- Papillomas in the **small duct** are **usually multiple** and located deeper within the ductal system.
- **Large papillomas** usually **present with nipple discharge**. Torsion on the stalk may produce infarction of the papilloma causing a bloody discharge. Most of the small duct papillomas present as small palpable masses, or detected as densities or calcifications on mammograms.

Proliferative Breast Disease with Atypia

This group includes **atypical ductal hyperplasia** and **atypical lobular hyperplasia**.

Proliferative breast disease with atypia:
Atypical epithelial hyperplasia.

MORPHOLOGY

Atypical hyperplasia is a cellular proliferation, which **resembles carcinoma in situ**.

Atypical hyperplasia:

- Cellular proliferation that resembles carcinoma in situ
- Ductal or lobular.
- **Atypical ductal hyperplasia:** It consists of a **monomorphic proliferation** of regularly spaced cells, sometimes with cribriform spaces. It resembles ductal carcinoma in situ (DCIS), but distinguished from it by being limited in extent and **only partially filling ducts**.
- **Atypical lobular hyperplasia:** It is a cellular proliferation identical to those of lobular carcinoma in situ (LCIS), but the **cells do not fill or distend more than 50% of the acini within a lobule**.

Clinical Significance of Benign Epithelial Changes (refer Box 24.1)

- Nonproliferative changes do not progress to cancer.
- Proliferative disease is associated with a mild increase in risk, while proliferative disease with atypia has a moderate risk of carcinoma.

CARCINOMA OF THE BREAST

Breast cancer:

- Most common cancer in women in the world
- Most common cancer in urban women in India
- Second most common cause of cancer-related death in women.

Carcinoma of the breast is the **second most common cancer in females**, first being carcinoma of cervix. Almost all breast carcinomas are adenocarcinomas.

Etiology

Q. Write short note on etiopathogenesis of breast carcinoma.

Risk Factors (Fig. 24.3)

Most important risk factor is gender and of breast cancer cases occur in only 1% of male.

1. **Age:** Breast cancer develops usually after the age of 25. Its incidence rises as the age advances and at 70 to 80 years and then declining slightly thereafter.
2. **Geographic variations:** They are observed and may be related to following:
 - **Type of diet:** Consumption of coffee (caffeine) may decrease the risk.
 - **Reproductive patterns:** These include number and timing of pregnancies.
 - **Nursing habits/breastfeeding:** Longer the women breastfeed, the greater the reduction in risk.
 - **Obesity:** Physical activity (exercise) may have a protective role.

Factors which reduce risk of breast carcinoma: Breastfeeding, exercise, healthy body weight.

3. **Race/ethnicity:** The variation in breast cancer risk genes across ethnic groups is in part responsible for racial or ethnic differences. For example, incidence of *BRCA1* and *BRCA2* mutations occur at different frequencies in different ethnic groups.
4. **Prolonged exposure to estrogens:** It increases the risk of breast carcinoma. It may be seen in the following conditions:
 - **Endogenous hormone exposure** occurs with long duration of reproductive life:

- **Early menarche** (<12 years) and **late menopause** (>55 years).
- **Late age at first-term pregnancy** (>35 years) and **nulliparity**.
- **Postmenopausal obese:** Increased risk due to the synthesis of estrogens in fat depots.
- **Carcinoma of the contralateral breast or endometrium:** Both produces prolonged estrogenic stimulation.
- **Exogenous hormone exposure:** It may be due to postmenopausal hormonal replacement therapy.

Carcinoma of breast: Long duration of breastfeeding has a protective effect.

Estrogens stimulate production of growth factors, which promote development of carcinoma.

Reduced estrogen decreases the risk of breast carcinoma.

5. **Germline mutations:** About 5–10% of breast cancers develop in women with germline mutations in tumor suppressor genes (refer pathogenesis below).
6. **Family history of first-degree relatives with breast cancer:** First degree relatives include mother, sister or daughter. It is strongly associated with increased risk for breast cancer. The risk is more if the relative had breast cancer at a young age or develop bilateral breast cancer.
7. **Environmental risk factors:**
 - **Radiation exposure:** Radiation to the chest due to cancer therapy, atomic bomb exposure, or nuclear accidents.
 - **Environmental toxins:** For example, organochlorine pesticides, have estrogenic effects.
 - **Cigarette smoking.**

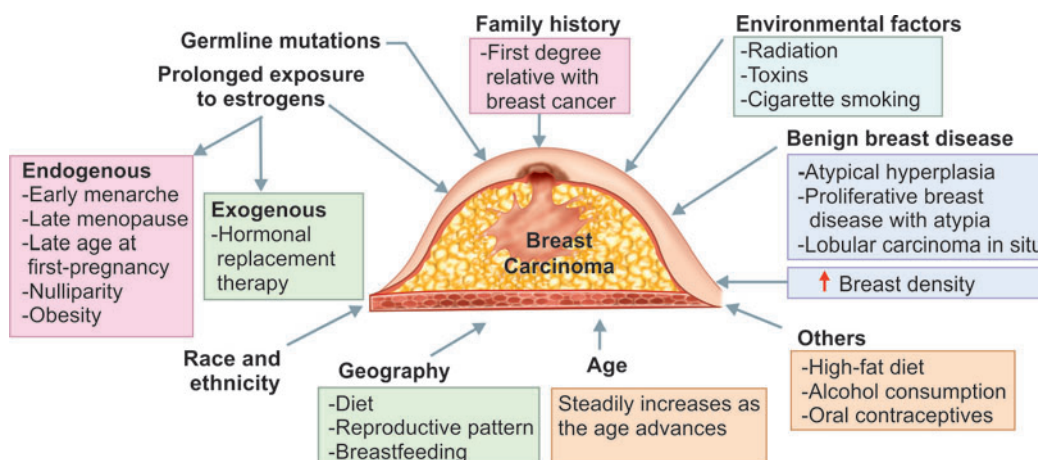


Fig. 24.3: Risk factors involved in the development of breast cancer

8. **Benign breast disease (Box 24.1): Atypical hyperplasia/proliferative breast disease with atypia/lobular carcinoma in situ.**

BOX 24.1: Epithelial pathologic lesion with relative risk of invasive breast cancer

- Nonproliferative breast changes (fibrocystic changes): 1%
- Proliferative disease without atypia: 1.5–2%
- Proliferative disease with atypia: 4–5%
- Carcinoma in situ: 8–10%

9. **Breast density:** Women with very dense breasts (high density) on mammography have a four- to six-fold higher risk of (both ER positive and ER negative) breast cancer compared to women with the lowest density. High breast radiodensity indicates deficient involution of lobules at the end of each menstrual cycle.
10. **Other possible factors:**
- High-fat diet
 - Moderate or heavy alcohol consumption
 - Oral contraceptives.

Pathogenesis (Fig. 24.4)

The pathogenesis of breast cancer is poorly understood. The major factors for the development of breast cancer are (1) genetic changes, (2) hormonal influences, and (3) environmental factors. Breast cancers represents clonal proliferations of cells with multiple genetic aberrations, which is influenced by hormonal exposures and inherited susceptibility genes.

- Breast carcinomas can therefore be divided into:
 - **Hereditary breast cancer:** Arises in women with germline mutations in tumor suppressor genes and environmental factors have a clear influence on its development.
 - **Sporadic breast cancers:** Both genetic and environmental factors contribute.

Detection of breast cancer susceptibility genes has helped in understanding the pathogenesis of both familial/hereditary and sporadic forms of breast cancer.

Familial/Hereditary Breast Cancer

- **Inheritance of susceptibility gene/s:** About 12% of breast cancers are due to inheritance of an identifiable susceptibility gene or genes. The evidences that favor hereditary etiology includes (1) multiple affected first-degree relatives, (2) early development of cancers, (3) multiple cancers, or (4) family members with other specific cancers. In some, one defective copy of a tumor suppressor gene inherited as an autosomal dominant trait. In these women, a single sporadic mutation in the remaining normal allele is will result in loss tumor suppressor function and is probably the initiating driver mutation.
- **Tumor suppressor gene:** The well-documented susceptibility genes for familial breast cancer are: *BRCA1*, *BRCA2*, *TP53*, and *CHEK2*. All these four are tumor suppressor genes and normally play a role in DNA repair and maintenance of genomic integrity. Complete loss-of-function of their gene products/proteins produces a “mutator” phenotype, an increased susceptibility to accumulate genetic damage that accelerates the development of breast cancer. **Mutations in *BRCA1* and *BRCA2* are responsible for 80–90% of “single gene” familial breast cancers and ~ 3% of all breast cancers.** *BRCA1* and *BRCA2* carriers are also have increased risk for other epithelial cancers (e.g. carcinoma of prostate and pancreas).

BRCA1 (on chromosome 17q21) and *BRCA2* (on chromosome 13q12.3) are large genes, and many mutations can occur throughout their coding regions.

- **Mutations in *BRCA1*** also greatly increase the risk of ovarian carcinoma (20–40%). *BRCA1*-associated breast cancers are usually poorly differentiated and show “medullary features” (i.e. syncytial growth pattern, pushing margins and a lymphocytic response refer page 703) and behave similar to ER-negative/HER2-negative breast cancers identified as “basal-like” by gene expression profiling.
- **Mutations in *BRCA2*** are associated with smaller risk for ovarian carcinoma (10–20%) but are associated more frequently with carcinoma of male breast.

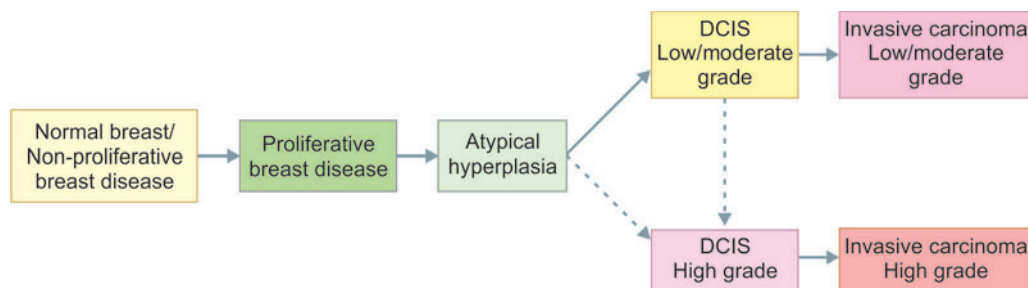


Fig. 24.4: Probable sequence of events in the development of breast carcinoma

BRCA2-associated breast carcinomas also poorly differentiated, but are mostly ER-positive than *BRCA1* cancers.

- **Others genes:** Germline mutations in *TP53* (Li-Fraumeni syndrome), mutations in *CHEK2*, *PTEN* (Cowden syndrome), *STK11* (Peutz-Jeghers syndrome), and *ATM* (ataxia telangiectasia).

Sporadic Breast Cancer

- **Hormonal exposure:** The major risk factors for sporadic breast cancer are discussed under risk factors. These are mainly related to **hormone exposure: (1) gender, (2) age at menarche and menopause, (3) reproductive history, (4) breastfeeding, and (5) exogenous estrogens.** Hormonal exposure stimulates growth of the breast during puberty, menstrual cycles, and pregnancy. This in turn increases the number of cells that can potentially give rise to a cancer.
- **Other environmental risk factors:** Include radiation exposure, and exposure to chemicals with estrogen-like effects.

Molecular Carcinogenesis

It is thought that resident breast tissue stem cells are the cell of origin for all breast cancers. The breast carcinomas represent manifestations of the complex genetic and epigenetic changes which drive carcinogenesis.

- **ER-positive, HER2-negative cancers (luminal):** They constitute 50–65% of breast cancers and are the most common subtype of breast cancer in women who inherit germline mutations in *BRCA2*. They may be also associated with gains of chromosome 1q, losses of chromosome 16q, and activating mutations in *PIK3CA* (encodes phosphoinositide-3 kinase (*PI3K* that is an important component of signaling pathways downstream of growth factor receptors). These genetic changes are usually also found in flat epithelial atypia and atypical ductal hyperplasia (precursor lesions for this subtype of breast cancer). ER-positive cancers are called “luminal,” because they closely resemble normal breast luminal cells in terms of their mRNA expression pattern.
- **HER2-positive cancers (HER2 enriched):** They arise through a pathway associated with amplifications of the *HER2* gene on chromosome 17q. They constitute about 20% of all breast cancers and may be either ER-positive or ER-negative. This is the most common subtype in patients with germline mutations in *TP53* (Li-Fraumeni syndrome).

- **ER-negative, HER2-negative cancers (basal-like):** They arise via a distinct pathway which is independent of ER-mediated changes in gene expression and *HER2* gene amplifications. They constitute ~15% of breast cancers, but are the most common tumor type in patients with germline *BRCA1* mutations. Sporadic tumors of this type usually show loss-of function mutations in *TP53*; mutations in *BRCA1* are uncommon. However, *BRCA1* may be silenced in sporadic tumors by epigenetic mechanisms. These tumors have a “basal-like” pattern of mRNA expression (genes that are expressed in normal myoepithelial cells).

Role of stromal cells: Neoplastic epithelial cells are dependent on interactions with stromal cells in the local microenvironment. The stroma consists of fibroblasts, blood vessels, lymphatics, inflammatory cells, and extracellular matrix. The role of stroma is not yet completely known. However, it is observed that cancers develop in the areas with increased mammographic density (due to increased fibrous stroma) suggests that stroma is both a marker of risk and biologically important for tumorigenesis. Focal changes in the stroma may create a microenvironment required for tumor development and growth. Angiogenesis and tumor-associated inflammation are commonly found with carcinoma even in in-situ stage.

Classification of Breast Carcinoma

Q. Write short note on classification of breast carcinoma.

Histological Classification (Box 24.2)

Broad classification depends on a combination of histological pattern and cytological characteristics.

More than 95% of malignant tumors of breast are adenocarcinomas.

- **Noninvasive carcinoma (carcinoma in situ):** It is characterized by the presence of **malignant epithelial cells within the ducts and lobules and has not penetrated the basement membrane.**
- **Invasive carcinoma (“infiltrating” carcinoma):** It is characterized by malignant cells that has **penetrated** through the **basement membrane** into stroma.

Origin: All breast carcinomas arise from cells in the terminal duct lobular unit.

Tumors with only HER2 positivity: High frequency of brain metastasis.

BRCA1 positive woman have 60% increased risk of breast carcinoma.

BOX 24.2: Classification of common breast tumors**Epithelial Tumors**

- A. Precursor lesions/Noninvasive carcinoma
 - 1. Ductal carcinoma in situ (DCIS)
 - 2. Lobular carcinoma in situ (LCIS)
- B. Invasive (infiltrating) breast carcinoma
 - 1. Invasive carcinoma of no special type (NST), the most common subtype of invasive carcinoma
 - 2. Invasive lobular carcinoma
 - 3. Special histologic types of invasive carcinoma
 - i. Medullary carcinoma
 - ii. Mucinous carcinoma (colloid carcinoma)
 - iii. Tubular carcinoma
- C. Papillary lesions
 - 1. Intraductal papilloma
 - 2. Intraductal papillary carcinoma
 - 3. Solid papillary carcinoma

Tumors of the Nipple

- 1. Nipple adenoma
- 2. Paget disease of nipple

Fibroepithelial Tumors

- 1. Fibroadenoma
- 2. Phyllodes tumor: Low grade and high grade

Clinical Patterns

- 1. Inflammatory carcinoma

Upper outer quadrant: Most common site for cancer

PRECURSOR LESIONS/NONINVASIVE CARCINOMA

Carcinoma in situ: DCIS and LCIS

Classification

It is subclassified as:

1. **Ductal** carcinoma in situ (DCIS)
2. **Lobular** carcinoma in situ (LCIS).

Carcinoma in situ was originally classified as ductal or lobular based on the resemblance of the involved spaces to normal ducts or lobules (acini).

Presently, these terms are based on differences in tumor cell biology; and “lobular” refers to carcinomas of a specific type, and “ductal” is used more generally for adenocarcinomas that have no other designation. Presently WHO (2012) classifies them as precursor lesions.

Ductal Carcinoma in Situ (DCIS; Intraductal Carcinoma)

Q. Write short note on ductal carcinoma in situ/intraductal carcinoma.

- DCIS consists of a **malignant cells limited to ducts and lobules** by the basement membrane. The myoepithelial cells are preserved.
- They usually **involve the small and medium-sized ducts**.
- DCIS can **spread throughout ducts and lobules**. Thus, it can produce extensive lesions involving an entire sector of a breast.

DCIS: Most common malignancy associated with calcifications.

MORPHOLOGY

DCIS is divided into two subtypes **depending on the architecture**:

- A. DCIS-comedo (high-grade) subtype
- B. Non-comedo DCIS
 - Solid
 - Cribriform
 - Papillary
 - Micropapillary

Majority of DCIS show a mixture of above patterns.

A. DCIS-comedo (High-grade) Subtype

Comedo DCIS: Most likely to result in a palpable abnormality in the breast.

- **Gross:** Cut section of the tumor shows **distended duct-like structures** containing **white, necrotic material**; similar in appearance to comedones (hence the term comedo).
- **Microscopy** (Fig. 24.5):
 - Ducts contain solid sheets of very **large, pleomorphic epithelial cells** having **pleomorphic, irregular hyperchromatic nuclei**. The **central areas** show **necrosis**.
 - **Central necrotic area** commonly undergoes **dystrophic calcification**.

B. Noncomedo DCIS

- In these tumors, the ducts contain a **monomorphic population of tumor cells** with **nuclear grades ranging from low to high**.
- Both the **tumor cells and nuclei** are **smaller and more regular** than those of the comedo type.
- **Necrosis is minimal or absent**.
- Architectural patterns of noncomedo DCIS:
 1. **Solid DCIS** (Fig. 24.6A): It shows **tumor cells completely filling the involved spaces**.
 2. **Cribriform DCIS** (Fig. 24.6B): It shows **spaces between the intraductal tumor cells** that are **evenly distributed and regular in shape** (cookie cutter-like).

3. **Papillary DCIS** (Fig. 24.6C): It shows **papillae with fibrovascular cores** and without the normal myoepithelial cell layer.
4. **Micropapillary DCIS** (Fig. 24.6D): It shows **bulbous protrusions without a fibrovascular core**.

Prognosis of DCIS: If untreated, women with small, low-grade DCIS may **develop invasive cancer**. Mastectomy for DCIS is curative for over 95% of patients.

Lobular Carcinoma in Situ (LCIS)

- Arises in TDLU
- Tends to be **bilateral**
- More **common in young women** and majority occurring before menopause.

DCIS:

- Low grade: Cribriform, papillary and micropapillary
- High grade: Solid and comedocarcinoma.

Bloody nipple discharge: Intraductal papilloma and ductal carcinoma.

Molecular Changes

E-cadherin: Responsible for cohesion of normal breast epithelial cells, and is expressed by both benign breast epithelium and ductal cancers.

- LCIS is associated with **mutations of the E-cadherin gene** which results in lack of adhesive molecule E-cadherin.
- Lack of E-cadherin in LCIS result in **rounded tumor cells** due to loss of attachment to adjacent cells.

- **Lack of E-cadherin expression confirms the lobular nature of neoplastic cells.**

LCIS: Loss of E-cadherin.

MORPHOLOGY

- Consists of **loose and noncohesive (dyscohesive) cells** having **oval or round, regular nuclei** and **small nucleoli**.
- Tumor cells **smaller and more monotonous** than in DCIS.
- Mucin-positive signet-ring cells are commonly present.
- Immunohistochemistry:
 - **Lack of E-cadherin.**
 - Almost always **expresses ER and PR.**
 - **Does not overexpress HER2/neu.**

INVASIVE (INFILTRATING) CARCINOMA

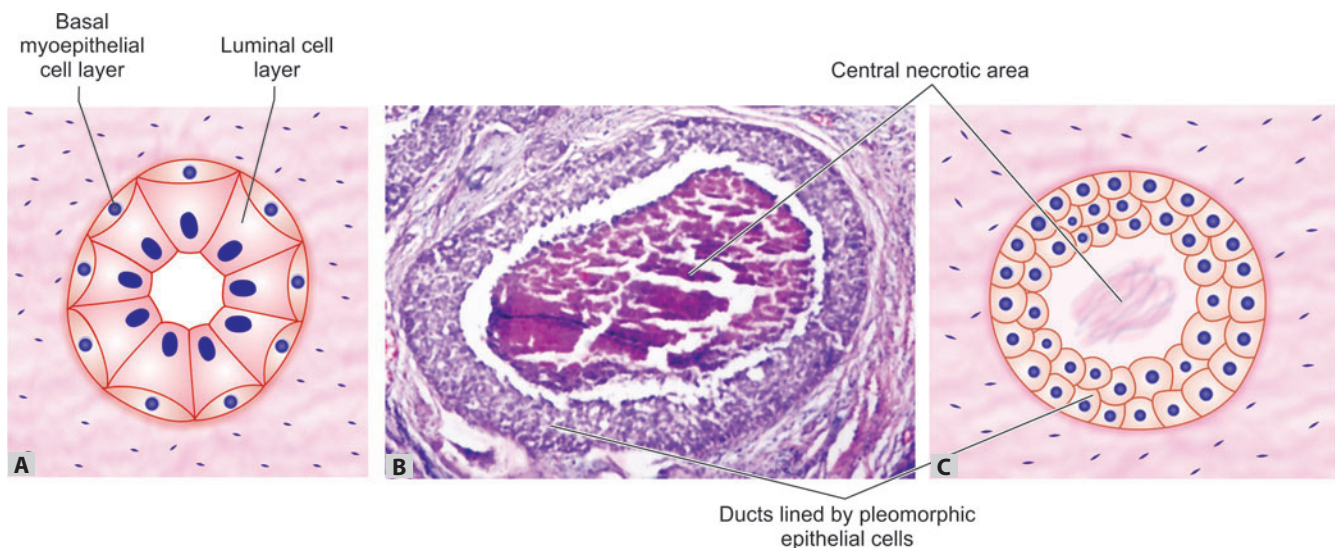
Invasive ductal carcinoma is the **most common histological type** of breast carcinoma.

Classification of Invasive Carcinoma

Invasive carcinomas can be divided depending on the molecular and morphological features.

Morphological Classification (refer Box 24.2)

- **No special type (NST):** Two-thirds are grouped together and called “ductal” or no special type (NST).
- **Special histological subtype:** About one-third of breast cancers are classified into special histologic types.



Figs 24.5A to C: (A) Normal duct/acinus lined by bilayered epithelium consisting of inner luminal cells and outer myoepithelial cells; (B) (photomicrograph); (C) (diagrammatic) DCIS-comedo subtype showing ducts containing large, pleomorphic epithelial cells and central area of the duct with necrosis

Molecular Classification

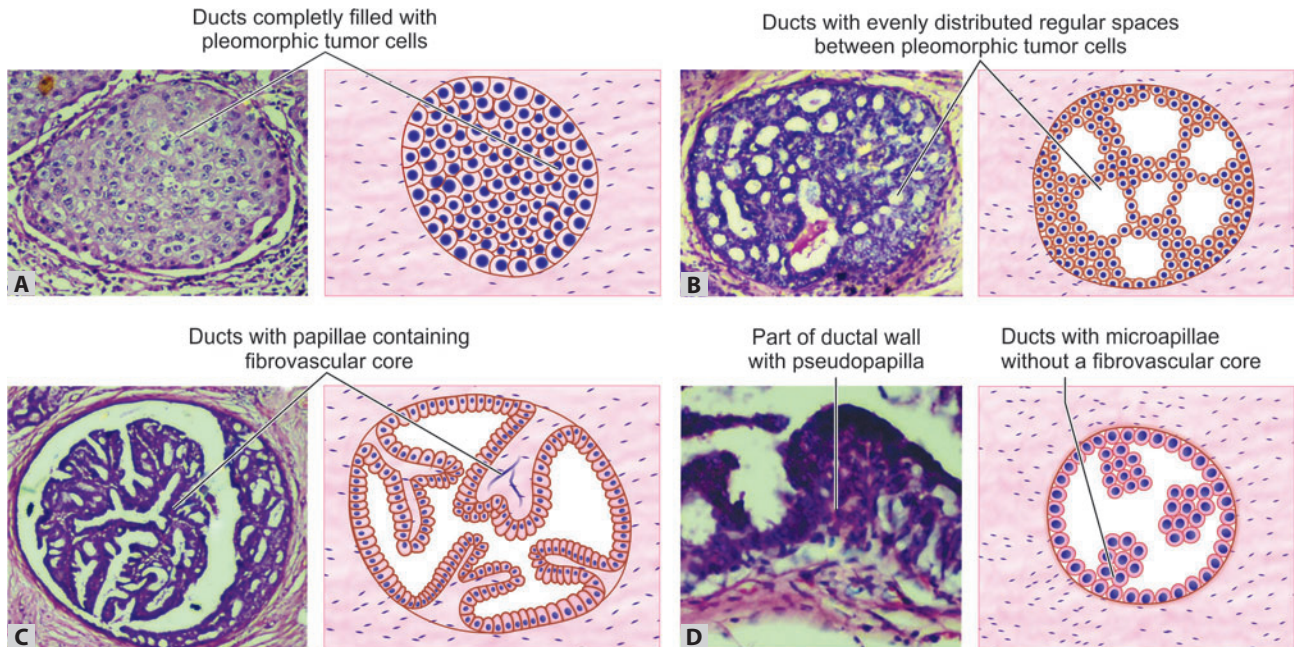
Based on the expression of estrogen receptor (ER) and HER2, invasive breast carcinoma can be divided into three major biologic subgroups (Table 24.1).

1. ER-positive, HER- negative (luminal)

Constitute 50–65% of cancers and is the most common molecular subtype of invasive breast cancer. It is

subdivided into two subgroups based on proliferation rates,

- **ER-positive, HER2-negative, low proliferation (40–55%):** These tumors form the majority of cancers in older women and in men. Many of them are detected at an early stage. The local recurrence rate is very low and is usually cured by surgery.



Figs 24.6A to D: Photomicrographic (left of each) and diagrammatic (right of each) appearances of noncomedo ductal carcinoma in situ (DCIS). (A) Solid; (B) Cribriform; (C) Papillary; and (D) Micropapillary type

TABLE 24.1: Molecular subtypes of invasive breast carcinoma

Features	ER-positive, HER2-negative (luminal)		HER2-positive (ER-positive or negative)	ER-negative HER2-negative (triple negative)
Percentage	~ 40–55 % (low proliferation)	~10% (high proliferation)	~20%	~15%
Special histologic types	Well/moderately differentiated lobular, tubular, mucinous	Poorly differentiated lobular	Some apocrine	Medullary
Type of patient	Older women, men	BRCA2 mutation carriers	Young women, TP53 mutation carriers (ER positive)	Young women, BRCA1 mutation carriers
Pattern of metastasis (percentage)	Bone (70%), visceral (25%) or brain (<10%)	Bone (80%), visceral (30%) or brain (10%)	Bone (70%), visceral (45%), and brain (30%)	Bone (40%), visceral (35%), brain (25%)
Complete response to chemotherapy	<10%	~ 10%	15% (ER positive) 30% (ER negative)	~30%
Recurrence	Late (>10 years)	Intermediate	Usually short (<10 years)	Usually short (<5 years)

Abbreviation: ER, Estrogen receptor.

- **ER-positive, HER2-negative, high proliferation (10%):** These tumors are ER-positive, but ER levels may be low and expression of progesterone receptor may be low or absent. It is the most common carcinoma associated with *BRCA2* germline mutations. There is higher expression of genes related to proliferation.

2. HER2-positive (~ 20%)

It is the **second most common molecular subtype of invasive breast cancer**. About 50% of them are ER-positive. When present, ER expression is usually low; progesterone receptor expression is usually absent. They are more common in young women. They are identified by assays of HER2 protein overexpression or HER2 gene amplification. They can metastasize early (to viscera and brain) and even when tumor is small in size. About, one-third or more of these patients respond completely to antibodies that bind and block HER2 activity, and have an excellent prognosis.

3. ER-negative, HER2-negative breast cancers (basal-like/triple negative carcinoma) (~ 15%)

They constitute the third major molecular subtype. They are more common in young premenopausal women. This forms the majority of carcinomas in women with *BRCA1* mutations. They have high proliferation and rapid growth. Hence, it present as a palpable mass. They share a number of genetic similarities with serous ovarian carcinomas (both types with germline *BRCA1* mutations. These can metastasize when small in size, frequently to viscera and to the brain. However, about 30% respond to chemotherapy and cure may be possible in this chemosensitive subgroup. Recurrences develop within 5 years of treatment. Local recurrence is common, even after mastectomy. Prolonged survival following distant metastasis is rare.

Common Features

Carcinoma of breast: Involvement of subdermal lymphatics (permeation and blockage/obstruction) of skin may produce changes in skin that mimic the appearance of an orange skin and termed **peau d'orange**.

- **Invasion:** It is characterized by the presence of tumor cells outside of the duct-lobular units and into the surrounding breast stroma.
- **Desmoplasia:** It is characterized by marked stromal fibrosis.

Clinical presentation

- Almost always produces a **palpable mass**; and in over 50% of patients are associated with axillary lymph node metastases.
- Invasion → **dimpling of the skin** or **fixation** of tumor to the underlying **chest wall**.
- **Retraction of the nipple** may occur when tumor involves the central portion of the breast.
- **Lymphatic involvement:** Involved lymphatics may block the local area of skin drainage and cause **lymphedema** and thickening of the skin.
- Mammographically, invasive carcinomas most commonly present as a radiodense mass.

Invasive (Infiltrating) Carcinoma, No Special Type (NST); Invasive Ductal Carcinoma

Invasive carcinoma NST: Firm, irregular and on cut section produce a characteristic grating sound similar to cutting an unripened pear/water chestnut).

- Invasive carcinomas of no special type **constitute about 70 to 80% of carcinomas**. **Larger carcinomas may infiltrate the pectoralis muscle and be fixed to the chest wall or invade into the dermis and produce dimpling of the skin**. If the tumor is in the central portion of the breast, it may cause retraction of the nipple. Rarely, breast cancer may first presents as metastasis to an axillary node or distant metastasis before cancer is detected in the breast. In these cases, the primary carcinoma may be small, or masked by dense breast tissue, or may not produce a desmoplastic response. This causes difficulty in detecting them by palpation or mammography.

MORPHOLOGY

Q. Write short note on morphology of invasive/infiltrating duct carcinoma of breast.

Gross (Fig. 24.7)

- **Firm to hard in consistency** and have an **irregular outline or border**.
- **Cut section** or scraping, they typically produce a characteristic **grating sound** (similar to cutting a water chestnut). The cut surface also **retracts below the surface**.
- **Dense fibrous stroma**.

Desmoplasia: Marked proliferation of stromal fibroblast in response to invasion by the tumor.

Microscopy (Fig. 24.8)**Invasive breast carcinoma are graded according to the Nottingham Histologic Score.**

Carcinomas are scored depending on (1) tubule formation, (2) nuclear pleomorphism, and (3) mitotic rate. The points are added and carcinomas into grade I, II and III.

- **Grade I (Well-differentiated carcinomas):** They show tumor cells forming **prominent tubule**. The tumor cells have small round nuclei, and mitotic figures are rare.
- **Grade II (Moderately differentiated carcinomas):** Tumor cells may form **tubules**, but they also show **solid clusters or single infiltrating cells**. The tumor cells show a greater degree of nuclear pleomorphism and also have mitotic figures.
- **Grade III (Poorly differentiated carcinomas):** They consist of **nests or solid sheets of cells**. The tumor cells also show enlarged irregular nuclei, numerous mitotic figures and areas of tumor necrosis.

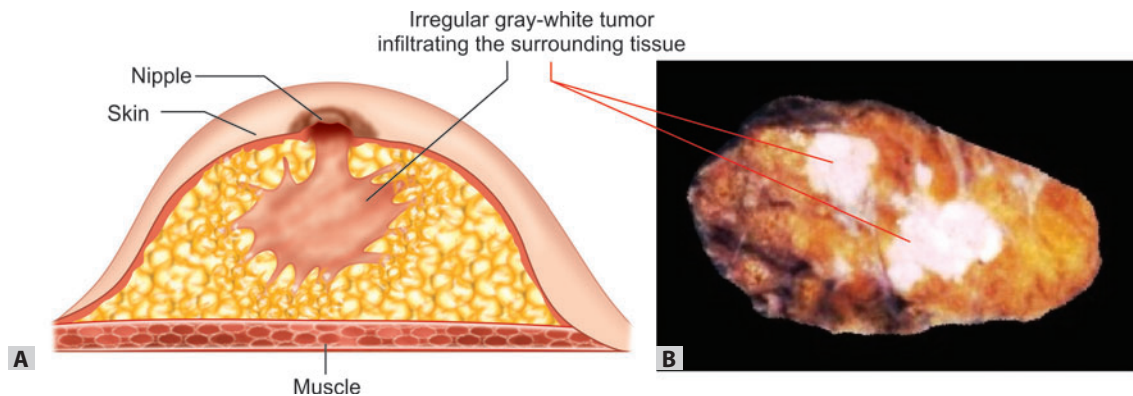
ER-positive, HER2-negative carcinoma: It may show different morphological patterns and grade may range from well to poorly differentiated. Mostly, they are well differentiated carcinomas. Mucinous, papillary, cribriform, and lobular patterns may be seen.

HER2-positive carcinoma: Most of them are poorly differentiated carcinomas and they have no specific morphological pattern. About 50% of apocrine carcinomas and 40% of micropapillary carcinomas belong to this group. It is more often associated with DCIS.

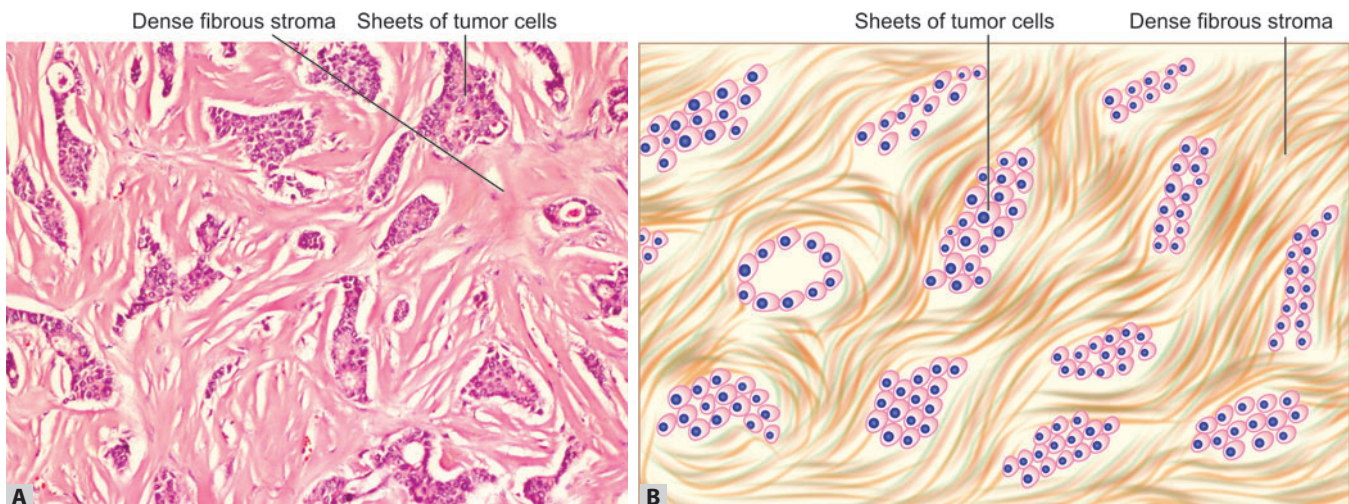
ER-negative, HER2-negative carcinomas: They are poorly differentiated and have circumscribed pushing borders with a central fibrotic or necrotic center. Tumors with similar appearance with a prominent lymphocytic infiltrate in the stroma (carcinomas with medullary features) also belong to this group.

NST: Histological grades

1. Well-differentiated
2. Moderately-differentiated
3. Poorly-differentiated.



Figs 24.7A and B: Gross appearance of invasive carcinoma of breast. (A) Diagrammatic; and (B) Mastectomy specimen with irregular, gray-white tumor



Figs 24.8A and B: (A) Hematoxylin and eosin (H & E); and (B) Diagrammatic. Microscopy of invasive carcinoma of breast showing irregular sheets of malignant cells separated by dense fibrous stroma (desmoplasia)

Special Histologic Types of Invasive Carcinoma

These are invasive carcinoma with distinctive/unique morphological, biologic, genetic and clinical features. Similar to “no special type”, of breast cancer, these special tumors can be grouped depending on expression of ER and HER2.

Medullary Carcinoma

Q. Write short note on medullary carcinoma of breast.

- **Age group:** Most common in the sixth decade.
- **Clinical presentation:** Presents as a **well-circumscribed mass** and mimics a benign lesion. It may present as a rapidly growing mass. Lymph node metastases are infrequent.
- Many medullary carcinoma are *BRCA1*-associated carcinomas and in about 67% of cases hypermethylation of the *BRCA1* promoter leading to downregulation of *BRCA1* expression is observed. However, germline *BRCA1* mutations is not found in majority of these tumors.

MORPHOLOGY

Medullary carcinoma of breast:

- Well circumscribed
- Syncytial sheets of tumor cells
- Large pleomorphic nuclei
- Minimal stroma infiltrated by lymphocytes and plasma cells.

Gross

- Medullary carcinoma usually **does not produce desmoplasia**. So, it is more yielding on palpation and cutting than typical breast carcinomas.
- The tumor is **soft, pale gray, fleshy** (medulla is Latin for “marrow”).
- **Well circumscribed**.

Microscopy (Fig. 24.9)

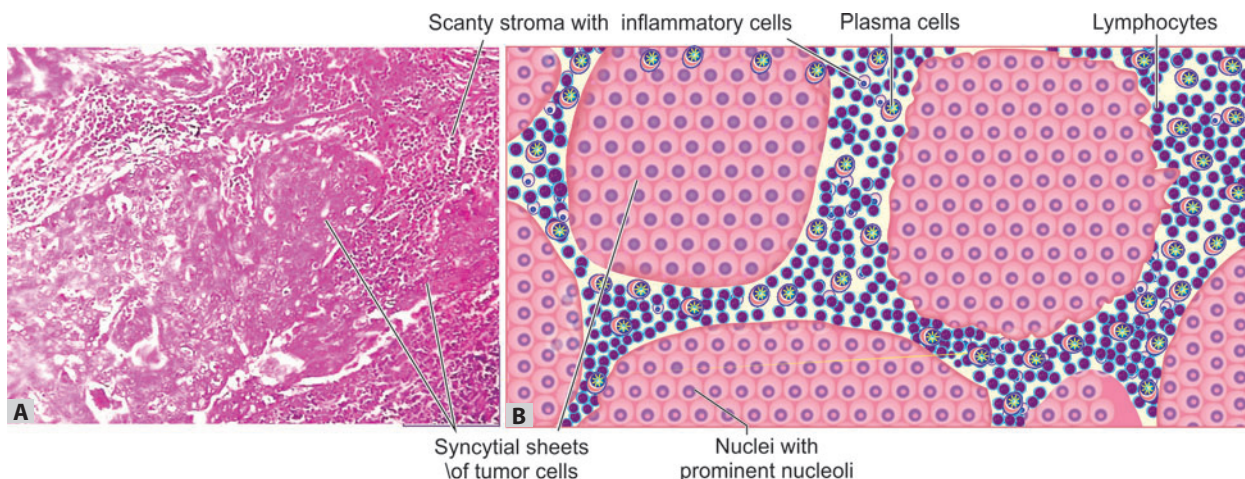
Medullary carcinomas are **poorly differentiated** and its features are:

1. **Tumor cells**
 - Arranged in **solid, syncytium-like sheets**.
 - Tumor cells are **large pleomorphic** with **vesicular, pleomorphic nuclei, and prominent nucleoli**.
 - **Mitotic figures are frequent**.
2. **Stroma:** **Minimal stroma** with moderate to marked **lymphoplasmacytic infiltrate**.
3. **Pushing (noninfiltrative) border**.

The syncytial growth pattern and pushing borders may be due to the **overexpression of adhesion molecules (e.g. E-cadherin)**, which can limit metastatic potential.

Prognosis

- Medullary carcinomas have a **slightly better prognosis** than NST carcinomas probably due to host immune response to tumor antigens (lymphocytes in stroma).
- HER2/neu overexpression is not observed.



Figs 24.9A and B: Medullary carcinoma of breast showing syncytial masses of large pleomorphic cells separated by stroma with lymphoplasmacytic infiltrate. (A) Photomicrograph and inset shows large pleomorphic tumor cells with prominent nucleoli; (B) Diagrammatic representation

Mucinous (Colloid) Carcinoma

Mucoid carcinoma:

- Elderly
- Slow growing
- Large amount of extracellular mucin
- ER positive
- Lymph node metastasis uncommon
- Better prognosis than NST or lobular carcinoma.
- Occur in older women
- Slow growing.

MORPHOLOGY

Gross

- Soft or rubbery
- Pale gray-blue gelatinous appearance
- Borders are pushing or circumscribed
- Cut section: Glistening surface and **muroid consistency**.

Microscopy

- Cuboidal to columnar tumor cells are arranged in clusters and small islands (occasionally forming glands).
- Background shows large amounts of extracellular mucin.
- **Molecular pathology:** They are usually diploid, and ER positive.
- **Lymph node metastases** are **uncommon**.
- **Prognosis:** Better than infiltrating ductal or lobular carcinoma.

Tubular Carcinoma

Tubular carcinoma of breast:

- Best prognosis
- Distant metastases are rare.
- Uncommon variant of invasive ductal carcinoma.
- Age group: Late 40s.

MORPHOLOGY

- **Gross:** Smaller than 1 cm in size and irregular.
- **Microscopy:** Very well differentiated and consist of **well-formed, angulated tubules** separated by dense stroma (Fig. 24.10).
- **Metastasis:** Rare.
- **Molecular pathology:** More than 95% are diploid, **ER positive**, and **HER2/neu negative**.
- **Prognosis:** Excellent.

Invasive Lobular Carcinoma

Q. Write short note on invasive lobular carcinoma of breast.

Most tumors show biallelic loss of expression of *CDH1* (gene encodes E-cadherin). Due to loss of E-cadherin,

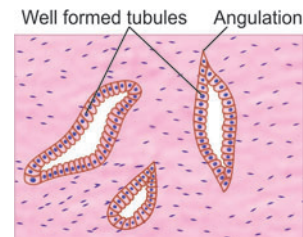


Fig. 24.10: Tubular carcinoma showing well-formed angulated tubules lined by single layer of tumor cells (diagrammatic)

it has discohesive cells and there is no desmoplastic response. They metastasize in characteristic patterns to the peritoneum and retroperitoneum, the leptomeninges (carcinomatous meningitis), the gastrointestinal tract, and the ovaries and uterus.

MORPHOLOGY

Gross

Invasive lobular carcinoma:

- Bilateral (more common)
- Multicentric
- Multifocal.

- Present as a palpable mass.
- Greater incidence of **bilaterality**.

Microscopy (Fig. 24.11)

Invasive lobular carcinoma:

- Small monotonous tumor cells
- Indian file arrangement
- Cytoplasmic mucoid globules
- Lack of E-cadherin.

- Shows **loose and non-cohesive (discohesive)** infiltrating tumor cells.
- Tumor cells are **smaller and monotonous with oval or round, regular nuclei and small nucleoli**.
- Tumor cells arranged in **single file (Indian file pattern)** or in loose clusters or sheets.
- **Signet-ring cells** containing mucin are common.
- Tubule formation is absent.
- Desmoplasia may be minimal or absent.

Metastasis

Unlike other breast cancers, lobular carcinomas **metastasizes** to the **peritoneum and retroperitoneum**, the **leptomeninges** (carcinoma meningitis), the **gastrointestinal tract**, and the ovaries and uterus.

Clinical Patterns

Inflammatory Carcinoma

Inflammatory carcinoma:

- **Most malignant** type of breast cancer
- Erythematous breast
- Mistaken for inflammatory condition
- Poor prognosis.

MORPHOLOGY

Gross

- Present as **swollen, erythematous** breast due to extensive invasion and obstruction of dermal lymphatics by tumor cells.
- Typically **does not form a discrete palpable mass**.
- **Mistaken as an inflammatory condition** and causes delay in diagnosis.
- **Many patients develop metastases at diagnosis and prognosis is poor.**

PAGET DISEASE OF THE NIPPLE

Q. Write short note on Paget disease of breast/nipple.

Paget disease of nipple is a **rare manifestation** (1 to 4% of cases) of **ductal carcinoma**, either in situ or invasive.

MORPHOLOGY

Gross (Fig. 24.12)

- **Skin of the nipple and areola** shows **ulceration** with oozing resembling eczema.
- **Underlying ductal carcinoma** (in situ or invasive).

Microscopy (Fig. 24.13).

Paget cells: Large spherical with clear cytoplasm and hyperchromatic nuclei. They represent extension from underlying ductal carcinoma via the lactiferous sinuses.

Skin lesions in the nipple shows **clusters of malignant cells** known as **Paget cells** in the **epidermis** without infiltrating the basement membrane. These cells represent extension from underlying ductal carcinoma via the lactiferous sinuses. The tumor cells allow seepage of extracellular fluid onto the nipple surface. The Paget cells can be detected by nipple biopsy or cytologic examination of the exudate.

- **Paget cells** are **large spherical cells** with **clear cytoplasm** and **hyperchromatic nuclei**.

Immunohistochemistry: Underlying carcinoma when present are usually poorly differentiated, **ER negative**, and **overexpress HER2/neu**.

Clinical Presentation

- Presents as a unilateral **erythematous eruption** in the region of nipple and areola with a scale crust.
- **Pruritus** (itching) is common, and the lesion may be **mistaken for eczema**.
- **Palpable mass** in the breast is present in 50–60% of women and shows an **underlying invasive carcinoma**.
- Majority of women **without a palpable mass** have only **DCIS**.

Prognosis: Depends on the features of the underlying ductal carcinoma.

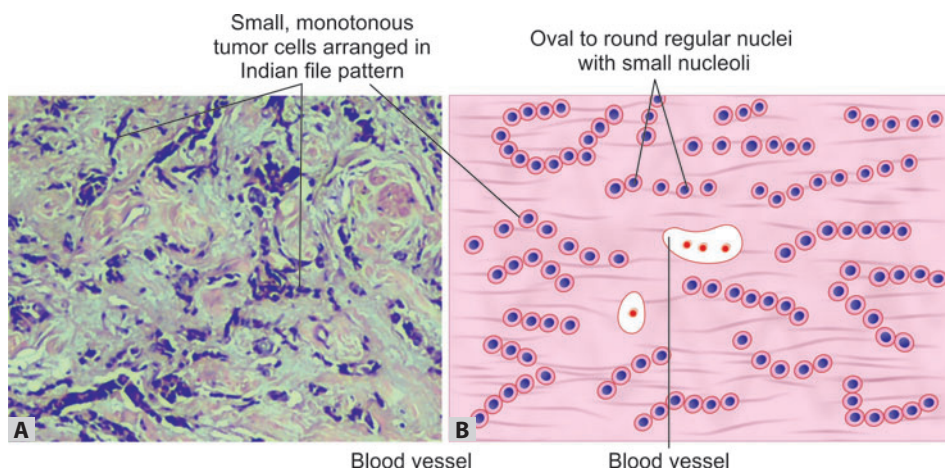
Pattern of lymphatic spread:

- Outer quadrant carcinoma to axillary lymph node
- Inner quadrant carcinoma to internal mammary lymph node.

SPREAD OF BREAST CARCINOMA

Breast carcinoma: Blood spread to lungs and bone.

Various routes of spread of breast cancer are mentioned in Table 24.2.



Figs 24.11A and B: (A) Photomicrograph and (B) Diagrammatic. Microscopic appearance of lobular carcinoma of breast showing small, monotonous loosely cohesive tumor cells arranged in Indian file pattern

Both lobular carcinoma of breast and signet ring carcinoma of GIT are characterized by the loss of E-Cadherin.

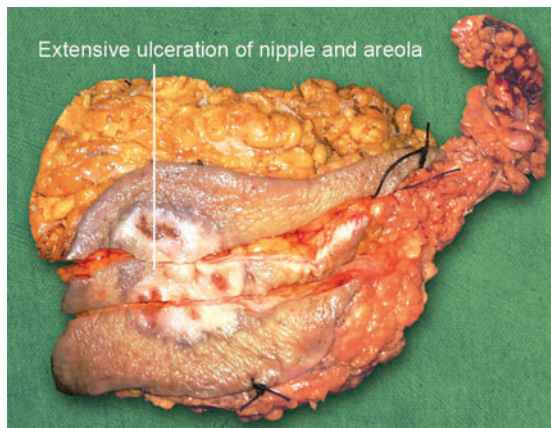


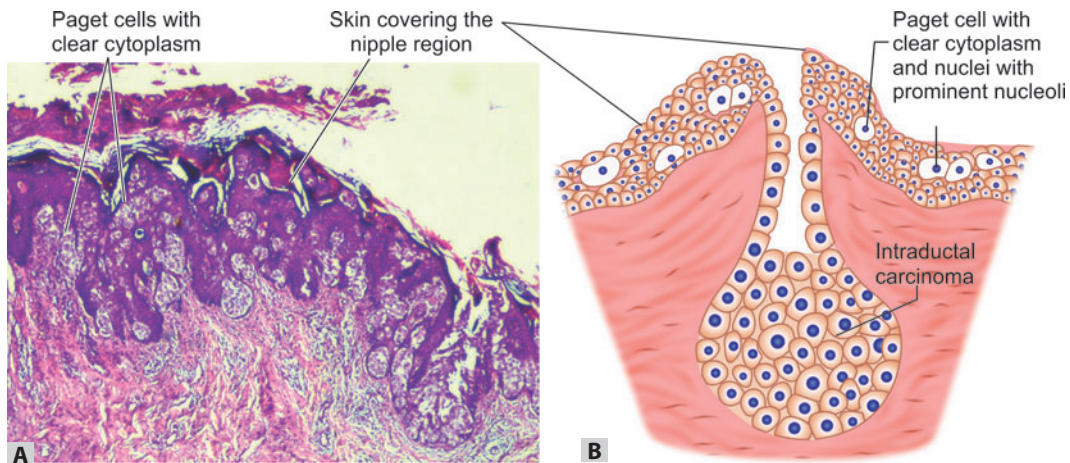
Fig. 24.12: Paget disease of nipple. Mastectomy specimen with extensive ulceration of nipple and areola

Paget disease of breast: Eczematous lesion of nipple associated with ductal carcinoma (in situ/invasive).

Histologic hallmark of Paget's disease of nipple is: Infiltration of the epidermis by malignant (Paget) cells.

Paget disease can also develop in extra-mammary sites, such as vulva.

Paget disease should not be mistaken for Paget disease of bone.



Figs 24.13A and B: (A) Photomicrograph; and (B) Diagrammatic shows microscopic features of Paget disease of breast

TABLE 24.2: Various routes of spread of breast carcinoma

Direct	Lymphatics	Hematogenous
Skin, including nipple and areola	Axillary lymph node	Lung
Chest wall	Internal mammary lymph node	Liver
	Supraclavicular lymph node	Brain
		Bone

PROGNOSTIC AND PREDICTIVE FACTORS (TABLE 24.3)

Q. Write short note on prognostic and predictive factors of breast carcinoma.

Prognosis is determined by the the biologic features of the carcinoma (molecular or histologic type) and the extent

TABLE 24.3: Prognostic and predictive factors of breast cancer

Major factors	Minor factors
Lymph node metastases	Histologic subtype
Tumor size	Histological grade
In situ versus invasive carcinoma	Estrogen and progesterone receptors (ER & PR)
Distant metastases	HER2/neu
Locally advanced disease	Lymphovascular invasion
Inflammatory carcinoma	Proliferative rate
	DNA content
	Response to neoadjuvant therapy
	Gene expression profiling

of cancer spread (stage) at the time of diagnosis. **Two important prognostic factors are tumor size and lymph node status.**

Prognostic factors can be divided into two groups:

1. Those related to the extent of carcinoma (stage) and
2. Those related to the underlying biology of the cancer.

Prognostic Factors Related to Extent of Carcinoma

1. **In situ versus invasive carcinoma:** Majority of **adequately treated DICS are cured**. About 50% of invasive carcinomas have metastases at the time of diagnosis.
2. **Distant metastases:** Cure is unlikely.
3. **Lymph node metastases:** **Status of the axillary lymph node is the most important prognostic factor** in the absence of distant metastases.

Sentinel node: Initial node draining the cancer.

- **Number and level involved** is directly related to the survival rate.
- Lymphatic vessels in most breast carcinomas drain first to one or two lymph nodes, which are known as **sentinel nodes**. These are **usually examined for metastasis**.
- Depending on the size of metastatic foci, they can be divided into:
 - **Macrometastases** means metastasis greater than 0.2 cm.
 - **Micrometastases** means metastasis 0.2 cm or less.
- Till recently, status of lymph node was considered as a major determinant of treatment choice. After the advent of molecular typing of carcinoma, the information obtained from nodal status is becoming less important. In the future, many women need not undergo sampling of nodes.

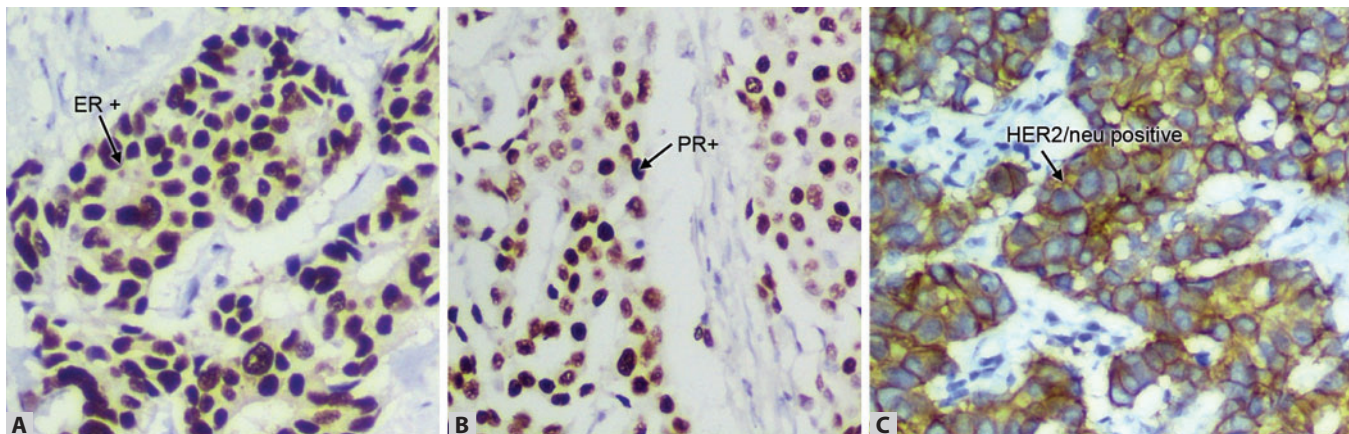
Most common site of metastasis from carcinoma of breast is: Bone (lumbar vertebra > femur > thoracic vertebra > rib > skull).

4. **Tumor size:** The risk of metastases to axillary lymph node increases with the size of the primary tumor. However, both are independent prognostic factors. Carcinoma of **less than 1 cm** in size without lymph node metastasis **have a 10-year survival rate of over 90%**, which drops to 77% for cancers more than 2 cm. Size is less important for HER2-positive and ER-negative carcinomas. Because they can metastasize even when tumor is quite small.

5. **Locally advanced disease:** Invasion into skin or skeletal muscle has a bad prognosis.
6. **Inflammatory carcinoma:** This is characterized by breast erythema and skin thickening. The edematous skin is tethered to the breast by Cooper ligaments and appears like the surface of an orange peel (peau d'orange). These signs are due to the filling of dermal lymphatics with metastatic carcinoma producing blockage of lymphatic drainage. It has poor prognosis. More than 60% of these cancers are ER-negative and 40 to 50% overexpress HER2.
7. **Lymphovascular invasion:** In about 50% of invasive carcinomas, tumor cells are found within vascular spaces (either lymphatics or small capillaries). This is strongly associated with lymph node metastases. It is a poor prognostic factor in cancers without lymph node metastases and is a risk factor for local recurrence. Severe plugging of the lymphovascular spaces of the dermis with carcinoma cells (inflammatory carcinoma) also has a very poor prognosis.

Prognostic Factors Related to the Underlying Biology of the Cancer

1. **Molecular subtype:** It is determined by immunohistochemistry for the expression of ER and HER2 and proliferation. It is an important prognostic factor.
2. **Special histologic type:** Survival rate for special types of invasive carcinomas (tubular, mucinous, medullary, lobular, and papillary) is greater than NST cancers.
3. **Histological grade: Nottingham histologic score** (also referred to as **Scarff-Bloom-Richardson**) is the **most commonly used grading system**, which classify invasive carcinomas into three groups (grade 1 to grade 3). These grades are highly correlated with survival. This grading system is **based on: 1) tubule formation, 2) nuclear grade and 3) mitotic rate**.
4. **Proliferative rate:** It can be measured by counting mitotic figures during histological grading or by immunohistochemical detection of proteins that are specifically expressed by actively dividing cells (e.g. cyclins, Ki-67). Proliferative rate is mainly important for ER-positive, HER2-negative carcinomas. Most of the ER-negative and/or HER2 positive carcinomas have high proliferative rates. Carcinomas with high proliferation rates have a poorer prognosis, but they may respond better to chemotherapy.
5. **Estrogen (ER) and progesterone receptors (PR):**
 - **ER and PR positive (Figs 24.14A and B):** These carcinomas **respond to hormonal manipulation**. ER-positive cancers are less likely to respond to chemotherapy.



Figs 24.14A to C: Immunohistochemistry in breast carcinoma. (A) ER positive; (B) PR positive and (C) HER2/neu positive

- **ER and PR negative:** These carcinomas are more likely to respond to chemotherapy.
- 6. **HER2/neu: Its overexpression** (Fig. 24.14C) is associated with **poorer survival**. However, they are predictor of response to agents that target this receptor.

Without adequate surgery, the majority of patients die with extensive local disease producing ulceration of the overlying skin. Carcinoma en cuirasse (literally “carcinoma of the breastplate”) is a complication should be prevented, even in patient with distant metastasis.

Triple assessment in breast cancer

1. Clinical examination
2. Radiological examination (mammography)
3. FNAC.

Breast cancer diagnosis: FNAC is a very useful, rapid, simple and economical procedure.

STROMAL/FIBROEPITHELIAL TUMORS

Q. Write short note on fibroadenoma of breast.

Two tumors that arise from intralobular stroma are **fibroadenoma** and **phylloides tumor**.

Fibroadenoma

Fibroadenoma: Most common breast tumor below 40 years of age.

Fibroadenoma: Benign tumor derived from intralobular stroma.

- **Most common benign tumor** of the female breast.
- **Age group:** Mostly occur in females between **20 to 30 years**.



Fig. 24.15: Fibroadenoma of breast showing a well-circumscribe tumor and cut surface grayish-white in color

- **Origin:** Arise from intralobular stroma.
- **Clinical presentation:** Young women usually present with a **palpable and freely movable mass**.

MORPHOLOGY

Gross (Fig. 24.15)

- Fibroadenomas can be single or multiple and unilateral or bilateral.
- **Spherical nodules** and are usually **well-circumscribed** and **freely movable**. The tumor can compress the surrounding breast tissue, but is not fixed. This accounts for its mobility on clinical examination → known as ‘**breast mouse**’.
- **Cut section:** It appears as **rubbery, glistening, grayish-white** nodules that bulge above the surrounding tissue and often contain **slit like spaces**.
- **Size:** Varies, usually **1 to 4 cm** in diameter.

Microscopy (Fig. 24.16)

Fibroadenoma: Mixture of duct-like structures separated by delicate fibrous connective tissue.

- Composed of a **mixture of duct-like structures** and **fibrous connective tissue**.
- **Duct-like structures:**
 - Ducts may be either simple and **round or elongate** and **branching**.

- Epithelium lining the ducts ranges from the **double layer of epithelium** of normal lobules to varying degrees of hyperplasia.
- **Fibrous connective tissue stroma:**
 - Constitutes most of the tumor
 - Stroma is **delicate, cellular**, often myxoid and resembles normal intralobular stroma.

Fibroadenoma:

1. Intracanalicular or
2. Pericanalicular type.

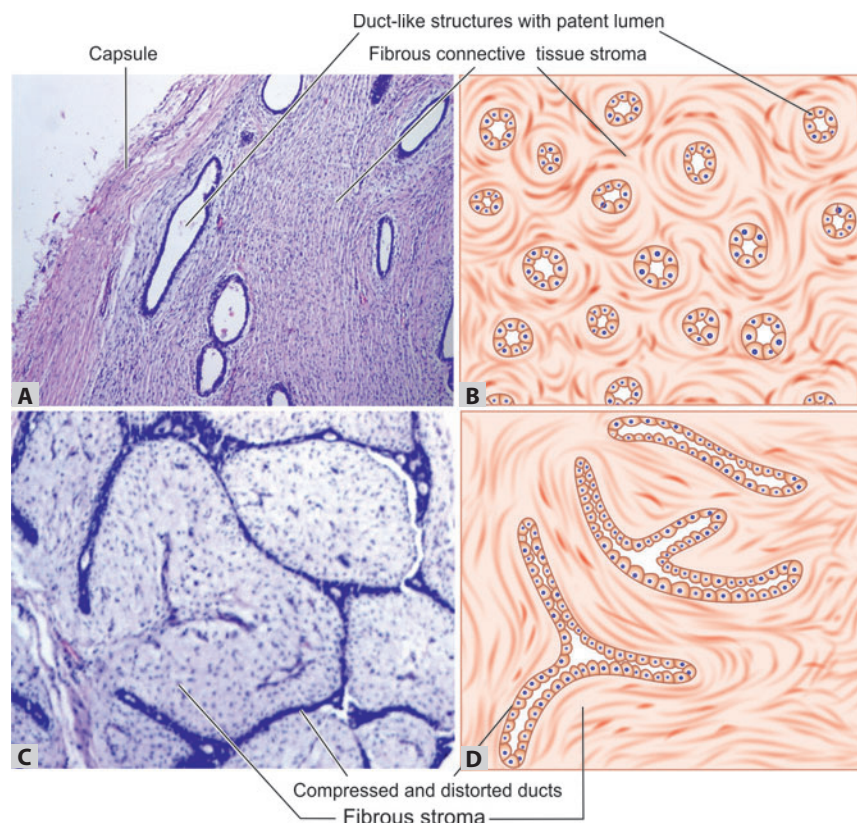
- **Classification:** According to the microscopic appearance:
 - **Pericanalicular** (Figs 24.16A and B): In this type, regular round or oval glandular configuration of the glands is maintained. The epithelium forms ducts with patent lumen, because the surrounding stroma proliferates circumferentially around them.
 - **Intracanalicular** (Figs 24.16C and D): It is a misnomer in which the connective tissue invaginates into the glandular spaces, so that it appears to be within them. The proliferated ducts are compressed and distorted by fibrous tissue reducing them to form curvilinear slits.

Usually both patterns co-exist.

- **Hormonal-induced changes:** Similar to normal breast, fibroadenomas can undergo hormonally-induced changes.
 - **Epithelial changes:** During pregnancy, fibroadenomas may grow rapidly in size; the glands may **increase in size** due to lactational changes.
 - **Stromal changes:** In older women (after menopause), the **stroma may become more fibrous and densely hyalinized**. It may also calcify and form large, lobulated (“popcorn”) calcifications.

MORPHOLOGIC VARIANTS OF FIBROADENOMA

1. **Tubular adenoma:** Well-circumscribed tumor composed of closely packed tubules with very scanty stroma.
2. **Lactating adenoma:** If an adenoma is composed of acini with secretory activity, it is seen during pregnancy or lactation (refer page 694).
3. **Juvenile fibroadenoma:** It is an uncommon variant of fibroadenoma that is larger (over 10 cm), and rapidly growing mass seen in adolescent girls. Microscopically, they are similar to fibroadenoma and does not recur after excision.



Figs 24.16A to D: Fibroadenoma of breast. (A) Hematoxylin and eosin (H & E); and (B) (diagrammatic) Pericanalicular type showing ducts with patent lumen, surrounded by delicate stroma. The border (left) shows sharp demarcation; (C) (H and E) and (D) (diagrammatic) Intracanalicular type composed of slit-like compressed ducts surrounded by fibrous tissue

Phyllodes Tumor

Q. Write short note on phyllodes tumor.

- **Definition:** Phyllodes tumor is a group of circumscribed **biphasic neoplasm** characterized by a double layered epithelial component arranged in clefts surrounded by an **hypercellular mesenchymal component** typically organized in a leaf-like structures.
- **Origin:** Arises from **intralobular stroma** (like fibroadenomas).

Phyllodes tumor: Grade depends on stromal cellularity.

- **Terminology:** Originally known as cystosarcoma phyllodes to imply its malignant behavior. Since, majority of these tumors behaved in a benign fashion, and most are not cystic, the **term phyllodes tumor is preferred**.
- **Age group:** Mostly occur **between 30 and 70 years of age**, with a peak in the fifth decade.
- **Clinical presentation:** Majority are detected as palpable masses.

MORPHOLOGY

Gross

- **Benign phyllodes tumor:** It is **round, sharply circumscribed**.
- **Malignant phyllodes:** It is usually poorly circumscribed and locally invasive with infiltrative borders.
- **Cut surface:** Solid, firm, glistening, gray-white bulging mass. It shows characteristic whorled pattern with **curved cleft-like spaces that resemble the leaf-buds** (phyllodes is Greek for "leaflike") Leaf-like appearance is due to the epithelium which covers the nodules of proliferating stroma.
- **Size:** Vary in size with an average size of about 5 cm in diameter.

Phyllodes tumor: Cut surface shows characteristic whorled pattern with curved cleft-like spaces that resemble the leaf-buds.

Microscopy (Fig. 24.17)

- **Growth pattern:** Typically show **exaggerated intracanalicular growth pattern with leaf-like projections into the dilated lumens**.
- **Two key features:** 1) presence of benign epithelial elements and 2) stromal hypercellularity.
 - **Benign epithelial component:** It consists of **luminal epithelial and myoepithelial cells**. They cover large club-like (bulbous)/leaflike projections (nodules) of proliferating stroma. In some tumors, these bulbous protrusions push or extend into a cystic space (hence the term cysto).
 - **Stromal hypercellularity:** It is the amount and appearance of the stromal component that determines biological nature of neoplasm.

- **Grading:** Depending on the appearance of the stromal component, phyllodes tumors are divided into (1) low-grade (benign) and (2) high-grade (malignant) phyllodes.
 - **Low-grade (benign) phyllodes:** It resembles fibroadenomas, but the stroma has following additional features:
 - ♦ **More cellular** (hypercellular) and resemble fibroblasts.
 - ♦ Contain **mitotic figures**.
 - **High-grade (malignant) phyllodes:**
 - ♦ **Hypercellular stroma**
 - ♦ **Abundant mitotic activity**
 - ♦ **Marked pleomorphism of stromal cells** like sarcomas (e.g. malignant fibrous histiocytoma, chondrosarcoma, rhabdomyosarcoma).
 - ♦ Majority of high-grade lesions show amplification of EGFR.

Recurrence

- Phyllodes tumors are likely to recur if not excised with wide margins.
- Low-grade tumors may recur locally but rarely metastasize.
- High-grade lesions frequently recur and may also develop hematogenous metastases. Metastatic deposits contain only the stromal component.

MALE BREAST

Male breast consists of the nipple and a rudimentary duct system without lobule formation.

Gynecomastia

Q. Write short note on gynecomastia.

Definition: Gynecomastia (enlargement of the male breast) is defined as the **enlargement of the male breast** due

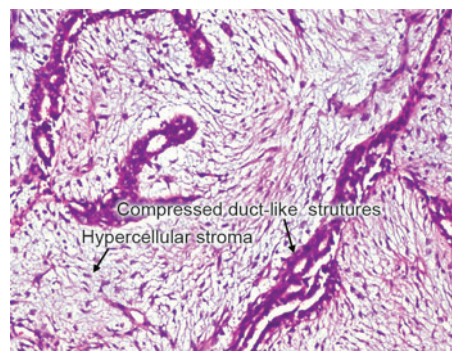


Fig. 24.17: Low-grade phyllodes tumor. Compared to a fibroadenoma, there is exaggerated intracanalicular growth pattern, increased stromal cellularity and overgrowth giving rise to the typical leaflike architecture

to hypertrophy and hyperplasia of both glandular and stromal components.

Etiology and Pathogenesis

Gynecomastia: Enlargement of the male breast due to hypertrophy and hyperplasia of both glandular and stromal components.

Male breast is subjected to hormonal influences similar to the female breast. Gynecomastia may occur due to an **imbalance between estrogens** (which stimulate breast tissue), **and androgens** (which counteract effects of estrogens).

- Gynecomastia before 25 years of age is usually due to hormonal changes during puberty.
- Gynecomastia during later years (any time during adult life)
 - **Hyperestrinism:** Cirrhosis and hormonally active tumors (Leydig cell tumor of testis, hCG-secreting germ cell tumors, lung carcinoma, or others).
 - **Drugs:** For example, alcohol, marijuana, heroin, antiretroviral therapy, anabolic steroids (used by some athletes and body builders), digitalis, reserpine, phenytoin, and some psychoactive agents.
 - Klinefelter syndrome (XXY karyotype).
 - Idiopathic.

MORPHOLOGY

Gross: Well-circumscribed, oval, disk-shaped mass of elastic consistency.

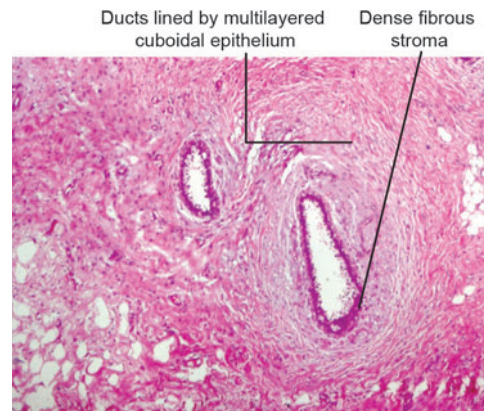


Fig. 24.18: Microscopic appearance of gynecomastia showing ducts are lined by a multilayered cuboidal epithelium surrounded by hyalinized fibrous tissue

Microscopy (Fig. 24.18)

- Ducts are lined by multilayered **columnar to cuboidal epithelium** with regular nuclei. The lining shows marked micropapillary epithelial hyperplasia.
- The ducts are surrounded by a **dense collagenous connective tissue stroma**.

Clinical Features

- Unilateral or bilateral.
- Usually centered below the nipple as a button-like subareolar enlargement, an important point in contrast to carcinoma, which tends to be located eccentrically.
- Advanced cases, it can simulate the adolescent female breast.

Endocrine Disorders

THYROIDITIS

Q. Describe the pathogenesis of Hashimoto thyroiditis.

Thyroiditis is a group of disorders characterized by **inflammation of the thyroid gland** (Box 25.1).

BOX 25.1: Various types of thyroiditis

1. Infectious thyroiditis
 - Bacterial including mycobacterial
 - Fungal
2. Hashimoto (chronic lymphocytic) thyroiditis
3. Granulomatous (subacute/de Quervain) thyroiditis
4. Reidel's thyroiditis

Hashimoto Thyroiditis

Hashimoto (chronic lymphocytic) thyroiditis: Most common cause of hypothyroidism in regions with adequate iodine levels.

- Hashimoto (**chronic lymphocytic**) thyroiditis is an **autoimmune disease** → gradual failure of thyroid function.
- **Age:** Peak between **45–65 years** of age.
- **Sex:** **More common in women** than in men. Female to male ratio 10: 1 to 20: 1.

Etiology

Hashimoto thyroiditis: First reported in 1912 by Hashimoto; goiter and intense lymphocytic infiltration of the thyroid (struma lymphomatosa).

Genetic Factor

- Hashimoto thyroiditis has a **strong genetic component** and is supported by the:
 - Concordance of disease in monozygotic twins (40%)
 - Presence of circulating antithyroid antibodies in asymptomatic siblings of Hashimoto patients
 - Association with other autoimmune diseases.
- **Genetic susceptibility:** Autoimmune disease such as Hashimoto and Graves disease are associated with **polymorphisms in genes associated with immune regulation** of T-cell responses such as cytotoxic T lymphocyte-associated antigen-4 (*CTLA4*) gene and *protein tyrosine phosphatase-22 (PTPN22)*.

Pathogenesis (Fig. 25.1)

Hashimoto thyroiditis: Autoimmune disease
Autoantibodies against

- Thyroglobulin
- Thyroid peroxidase.

Hashimoto thyroiditis is **autoimmune disease** caused by a breakdown in self-tolerance to thyroid autoantigens. It is characterized by the presence of circulating **autoantibodies against thyroglobulin and thyroid peroxidase**.

- **Failure of self-tolerance** to thyroid autoantigens is the initial event. Probably, it is abnormalities of regulatory T-cells (Tregs), or exposure of normally sequestered thyroid antigens.
- **Induction of thyroid autoimmunity:** It is the next event producing circulating autoantibodies against thyroid antigens.

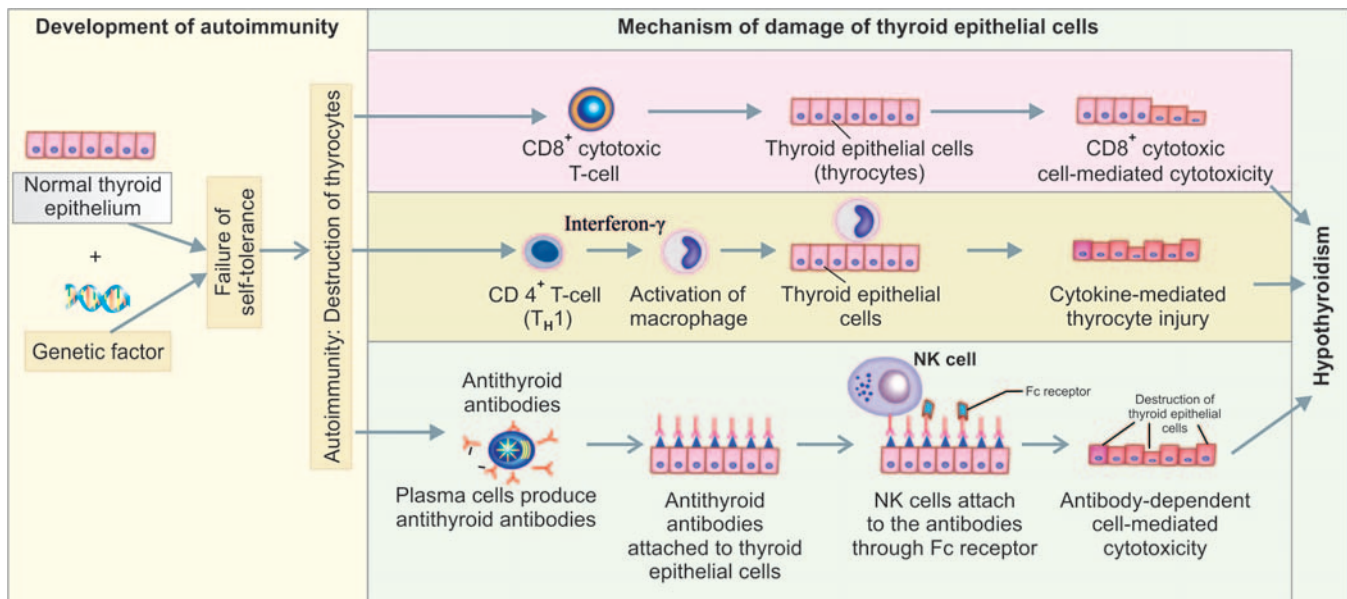


Fig. 25.1: Pathogenesis of Hashimoto thyroiditis. Failure of self-tolerance to thyroid autoantigens, results in progressive autoimmune destruction of thyroid epithelial cells by infiltrating cytotoxic T-cells, through cytokine or by antibody-dependent cell-mediated cytotoxicity

- **Progressive destruction of thyrocytes (thyroid epithelial cells):** It is accompanied by replacement of the thyroid parenchyma by mononuclear cell infiltration and fibrosis.

Hashimoto thyroiditis-mechanism of damage:

1. T-cell mediated cytotoxicity (type IV hypersensitivity)
2. Cytokine-mediated cytotoxicity
3. Antibody dependent cell-mediated cytotoxicity (type II hypersensitivity).

Mechanism of Thyrocyte Death

- Immunologic mechanisms causing death of thyrocytes in the thyroid follicle may be brought out by:
 1. **T-cell-mediated cell death:** CD8⁺ cytotoxic T-cells are main mediators responsible for thyrocyte destruction (type IV hypersensitivity).
 2. **Cytokine-mediated cell death:** Proliferation of CD4⁺ T-cells (T_H1 cell) produces inflammatory cytokines such as interferon-γ in the thyroid gland. These cytokines recruit and activate macrophages → damages thyrocyte.
 3. **Antibody-dependent cell-mediated cytotoxicity:** Antithyroid antibodies (antithyroglobulin and antithyroid peroxidase antibodies) bind to the antigens on the thyrocyte. NK cells bind to these antibodies through Fc receptor causing antibody-dependent cell-mediated cytotoxicity (type II hypersensitivity).

MORPHOLOGY

Q. Describe the morphological changes in Hashimoto thyroiditis.

Gross (Fig. 25.2)

- Diffuse and symmetric enlargement of thyroid gland.
- Gland is firm and nodular.
- Capsule is intact, and the thyroid gland is well-demarcated from adjacent structures.
- Cut surface is pale, gray-tan and shows accentuation of normal lobulation.

Microscopy (Fig. 25.3)

Hashimoto thyroiditis:

- Destruction of thyroid parenchyma
- Hürthle cell metaplasia
- Lymphoplasmacytic infiltrate.

1. Inflammation:

- Dense mononuclear inflammatory infiltrate consisting of small lymphocytes and plasma cells in the thyroid parenchyma.
- Lymphoid follicles with well-developed germinal centers.

2. Epithelial changes:

- **Atrophy of thyroid follicles:** They appear smaller than normal follicles.
- **Hürthle cell metaplasia:** It is a metaplastic response of the follicular epithelium to injury. Hürthle cells (Askanazy/oxyphil cells or oncocytes) have abundant eosinophilic, granular cytoplasm and line some of the follicles. Ultrastructurally, they have prominent mitochondria.

3. **Fibrosis:** The **interstitial connective tissue** is increased (**fibrosis**) and may cause atrophy of thyroid follicles. In contrast to Reidel thyroiditis, the **fibrosis does not extend beyond the capsule** of the gland.

Hürthle cell metaplasia:

- Metaplastic response to injury
- Abundant eosinophilic, granular cytoplasm
- Prominent mitochondria.

Hashimoto thyroiditis: Fibrosis does not extend beyond the capsule of the gland.

Fine-needle aspiration cytology (FNAC): It shows Hürthle cells with a heterogeneous population of lymphocytes.

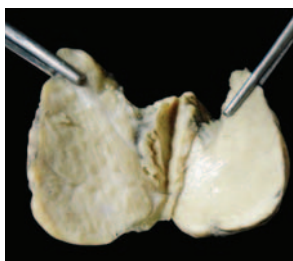


Fig. 25.2: Gross appearance of Hashimoto thyroiditis. Cut section shows enlargement of thyroid, pale gray-tan color and accentuation of normal lobulation

Risk in Hashimoto thyroiditis: Development of

1. Other autoimmune diseases
2. B-cell non-Hodgkin lymphomas (e.g. MALT lymphomas)
3. Papillary carcinomas.

Clinical Course

- **Painless enlargement of the thyroid** in middle-aged woman
- **Hypothyroidism** gradually develops
- Early stages may produce transient thyrotoxicosis due to destruction of thyroid follicles, with secondary release of thyroid hormones (Hashitoxicosis).

Subacute (Granulomatous) Thyroiditis

- Subacute (de Quervain) thyroiditis is **less common** than Hashimoto disease
- **Age:** Common between **40 and 50 years** of age
- **Sex:** Affects **women** more often than men (4: 1).

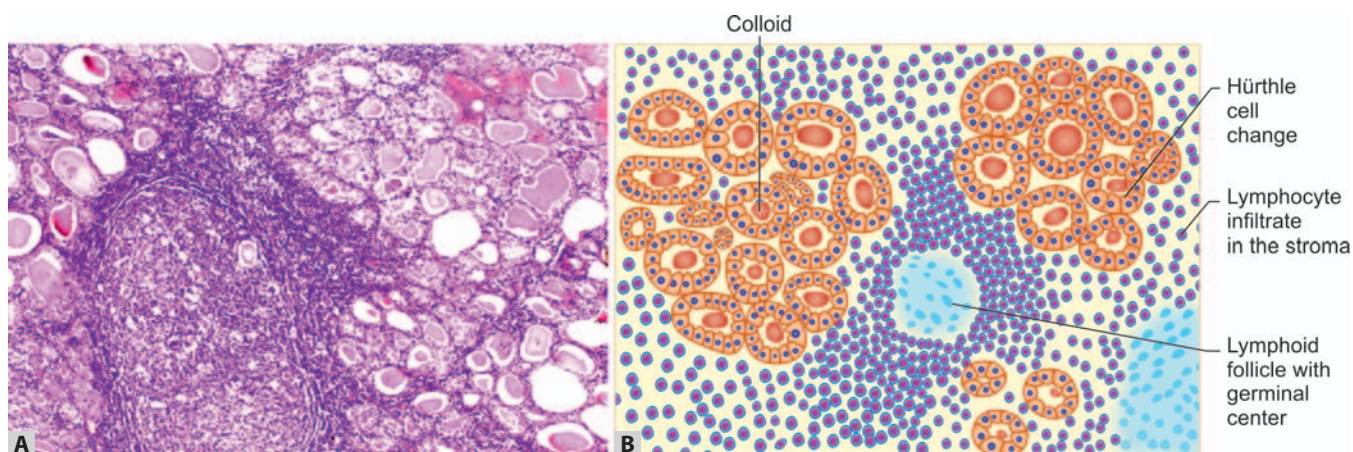
Etiology and Pathogenesis

Subacute thyroiditis is thought to be **initiated by a viral infection**. Points in favor are:

- Majority have an upper respiratory infection just before the onset of thyroiditis
- Seasonal variation with peak occurrence in the summer
- Association with virus infection (e.g. coxsackie virus, mumps, measles, adenovirus).

Pathogenesis

- Pathogenesis is not known
- Probably viral infection exposes a viral or thyroid antigen, which is released secondary to virus-induced host tissue damage



Figs 25.3A and B: (A) Photomicrograph; (B) Diagrammatic. Microscopy of Hashimoto thyroiditis shows parenchyma densely infiltrated by lymphocytes with germinal centers. Some of the thyroid follicles lined by deeply eosinophilic Hürthle cells

- This antigen stimulates cytotoxic T lymphocytes, which damage thyroid follicular cells
- The immune response is limited and does not progress.

MORPHOLOGY

Subacute thyroiditis: Granulomatous reaction against colloid escaped from damaged follicles.

Gross

- **Unilateral or bilateral enlargement** of the thyroid gland
- **Cut section:** The involved regions are **firm and yellow-white**.

Microscopy

1. **Damaged thyroid follicles** with escape of colloid.
2. **Inflammation:** It is elicited by the escaped colloid seen surrounding the damaged follicles. Consists of aggregates of **lymphocytes, activated macrophages, and plasma cells**.
3. **Granulomatous reaction:** It develops as lesion progresses and shows **multinucleate giant cells surrounding** pools or fragments of **colloid**. Hence, the designation **granulomatous thyroiditis**.
4. **Fibrosis:** It develops at late stages replacing the destroyed area of the gland.

Clinical Course

- **Painful enlargement** of the thyroid.
- **Early phase: Hyperthyroidism** with high serum T_4 and T_3 levels and low serum TSH levels
- **Recovery within 6–8 weeks** and thyroid function returns to normal.

Riedel Thyroiditis

- Less common form of thyroiditis.
- **Etiology:** It is **unknown**, but the presence of circulating antithyroid antibodies in most patients **suggests** an **autoimmune** etiology.

- **Gross:** Thyroid is **stony hard and fixed** which clinically simulates a thyroid carcinoma.

Reidel's thyroiditis: Fibrous tissue replacement of gland and surrounding tissue.

- **Microscopy:** Shows **extensive fibrosis** involving the **thyroid and contiguous neck structures**.

THYROTOXICOSIS

Thyrotoxicosis: Excessive levels of thyroid hormone from any cause.

Definition: Thyrotoxicosis is a **systemic syndrome** (with hypermetabolic state) caused by exposure to **excessive levels of thyroid hormone** (free T_3 and T_4).

Causes of Thyrotoxicosis (Box 25.2)

Hyperthyroidism: Thyrotoxicosis due to excessive synthesis of thyroid hormone.

BOX 25.2: Causes of thyrotoxicosis

Associated with hyperthyroidism

Primary hyperthyroidism

- Graves' disease (diffuse toxic hyperplasia)
- Toxic multinodular goiter
- Toxic adenoma

Secondary hyperthyroidism

- TSH-secreting pituitary adenoma (rare)

Not associated with hyperthyroidism

- Granulomatous (de Quervain) thyroiditis
- Struma ovarii (ovarian teratoma with ectopic thyroid)

Thyrotoxicosis Associated with Hyperthyroidism

It is the **clinical consequence due to the excessive circulating thyroid hormone (excessive thyroid function/hyperfunction)**. It is the **most common cause of thyrotoxicosis**.

Causes of Hyperthyroidism

Excessive synthesis and secretion of thyroid hormone may be primary disorder of thyroid or secondary to other disorders.

- **Primary hyperthyroidism:** It is due to an intrinsic thyroid abnormality.
 - **Abnormal thyroid stimulation:** Diffuse hyperplasia of the thyroid associated with **Graves' disease**.
 - **Intrinsic disease of the thyroid gland.**
 - ♦ Toxic multinodular goiter
 - ♦ Toxic adenoma of the thyroid.
- **Secondary hyperthyroidism:** It is due to processes arising outside of the thyroid, such as increased TSH-secreting **pituitary adenoma** (rare).

Thyrotoxicosis not Associated with Hyperthyroidism

Excessive release of **preformed thyroid hormone** from **thyroid** (e.g. in thyroiditis) or an **extrathyroidal source** (e.g. struma ovarii).

Clinical Manifestations of Hyperthyroidism

- Due to the **hypermetabolic state** produced because of excess of thyroid hormone and to **overactivity of the sympathetic nervous system** (i.e. an increase in the

β -adrenergic “tone”). Excessive thyroid hormone results in an **increase in the basal metabolic rate**.

- **Skin:** Soft, warm and moist. Heat intolerance and sweating.
- **Cardiac manifestations:** Increased cardiac output, tachycardia, palpitations, arrhythmias and cardiomegaly.
- **Neuromuscular system:** Fine tremor, hyperactivity, nervousness, anxiety, emotional lability, inability to concentrate, and insomnia.
- **Ocular changes:** Lid retraction → causes a staring appearance. However, **Graves’ disease is associated with proptosis** that comprises Graves’ ophthalmopathy.
- **Gastrointestinal system:** Increased stool frequency, often with diarrhea.
- **Skeletal system:** Osteopenia and a small increase in fracture rate.
- **Thyroid storm:** It is the sudden onset of severe hyperthyroidism. It occurs most commonly in Graves’ disease and probably due to sudden elevation in catecholamine levels.

Diagnosis of Hyperthyroidism

Hyperthyroidism: Serum TSH is the most useful screening test.

- **Clinical findings.**
- **Laboratory findings:**
 - **Serum TSH concentration:** It is the **most useful single screening test** for hyperthyroidism, because it is **decreased even at subclinical stage**.
 - **Free T₄:** It is **increased**.
 - Measurement of **radioactive iodine uptake** by the thyroid gland to determine the cause. For example, diffusely increased uptake in the whole gland is seen in Graves’ disease, increased uptake in a solitary nodule in toxic adenoma, or decreased uptake in thyroiditis.

GRAVES’ DISEASE

Q. Describe the pathogenesis of Graves’ disease.

Graves’ disease (also known as Basedow disease) is the **most common cause of hyperthyroidism**.

Graves’ disease: Most common cause of endogenous hyperthyroidism and thyrotoxicosis.

Triad of Clinical Findings

Triad of Graves’ disease:

1. Hyperthyroidism
2. Exophthalmos
3. Pretibial myxedema.

1. **Hyperthyroidism:** It is due to diffuse hyperplasia of the thyroid.
2. **Infiltrative ophthalmopathy** → results in exophthalmos.
3. Localized, infiltrative **dermopathy** (pretibial myxedema) in few patients.

Age: Peak between **20 and 40 years** of age.

Sex: **Females** are affected 10 times **more frequently** than males.

Etiology

Graves’ disease: First reported by Graves as “violent and long continued palpitations in females” associated with enlargement of the thyroid gland.

Graves disease (hyperthyroidism) and **Hashimoto thyroiditis** (hypothyroidism) are considered as **two extremes of autoimmune thyroid disorders**.

Genetic Factor

- Graves’ disease has a **strong genetic component** and is supported by the following observations:
 - Concordance of disease in **monozygotic twins** (60%).
 - **Presence of circulating antithyroid antibodies** in asymptomatic siblings of Hashimoto patients.
 - **Association with other autoimmune diseases.**
- Genetic susceptibility for Graves’ disease has been associated with polymorphisms in **genes associated with immune regulation**, e.g. cytotoxic T lymphocyte-associated antigen-4 (*CTLA4*) gene and protein tyrosine phosphatase-22 (*PTPN22*).

Pathogenesis (Fig. 25.4)

Graves’ disease: Autoimmune disease caused by autoantibodies most importantly against the TSH receptor.

Graves’ disease is an **autoimmune disease** characterized by the **presence of multiple autoantibodies most importantly against the TSH receptor**.

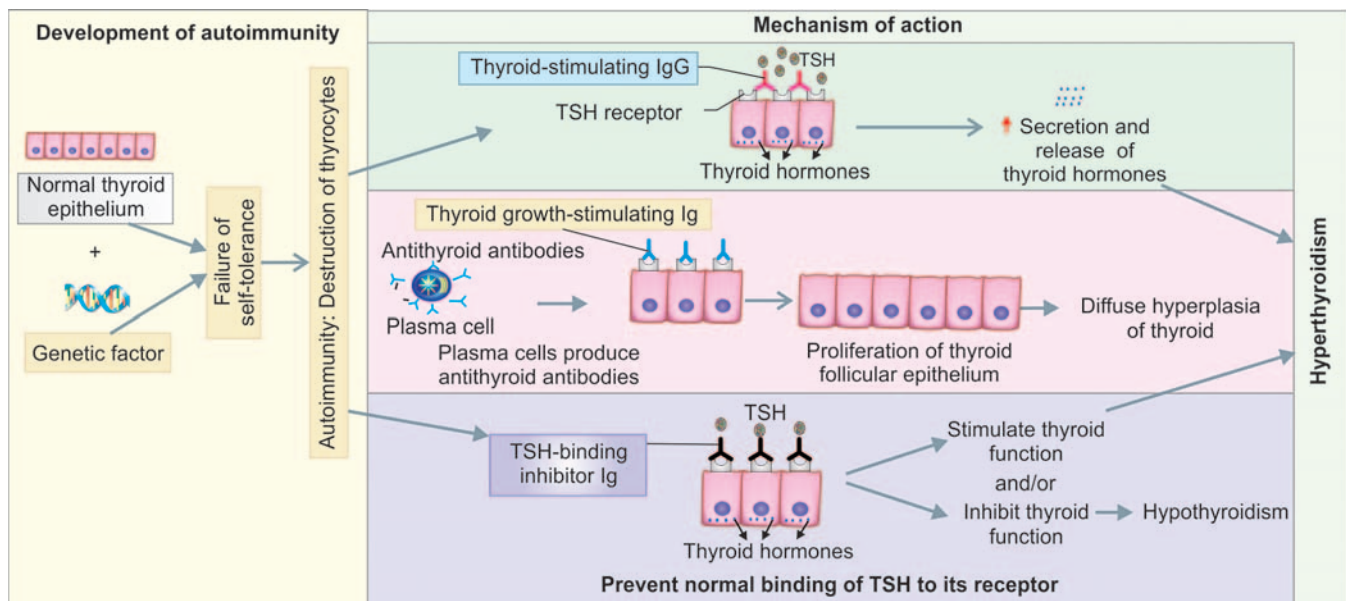


Fig. 25.4: Pathogenesis of Graves' disease

- **Failure of self-tolerance** to thyroid autoantigens is the initial event
- **Production of multiple autoantibodies.**

Autoantibodies in Graves' Disease

Autoantibodies in Graves' disease:

1. Thyroid-stimulating Ig
2. Thyroid growth-stimulating Ig
3. TSH-binding inhibitor Ig (anti-TSH receptor antibodies).

1. **Thyroid-stimulating immunoglobulin:** It is an immunoglobulin (Ig) G antibody, which binds to the TSH receptor on the plasma membrane of thyrocytes.
 - **Characteristics:** (1) Almost all patients show this autoantibody and (2) It is **specific** for Graves' disease.
 - **Action:** They act as agonists and stimulate the TSH receptor and **mimics the action of TSH** → **increases** the secretion and release of **thyroid hormones** (refer Fig. 6.6).

Graves' disease: Anti-TSH receptor antibody-type II hypersensitivity reaction.

2. **Thyroid growth-stimulating immunoglobulin:** It is also directed **against the TSH receptor**.
 - **Action:** Causes **proliferation of thyroid follicular epithelium** → **diffuse hyperplasia** of the thyroid gland.

3. **TSH-binding inhibitor immunoglobulin (anti-TSH receptor antibody):** It **prevents normal binding of TSH to its receptor** on thyroid epithelial cells.

- **Action:** Varies.

- **Stimulation:** Some forms **mimic** the action of TSH → stimulate thyroid epithelial cell causing hyperthyroidism.
- **Inhibition:** Some forms may **inhibit** thyroid cell function → hypothyroidism.
- **Coexistence of stimulating and inhibiting immunoglobulins:** This action in the same patient may → responsible for episodes of hypothyroidism in some patients with Graves' disease.

Infiltrative Ophthalmopathy

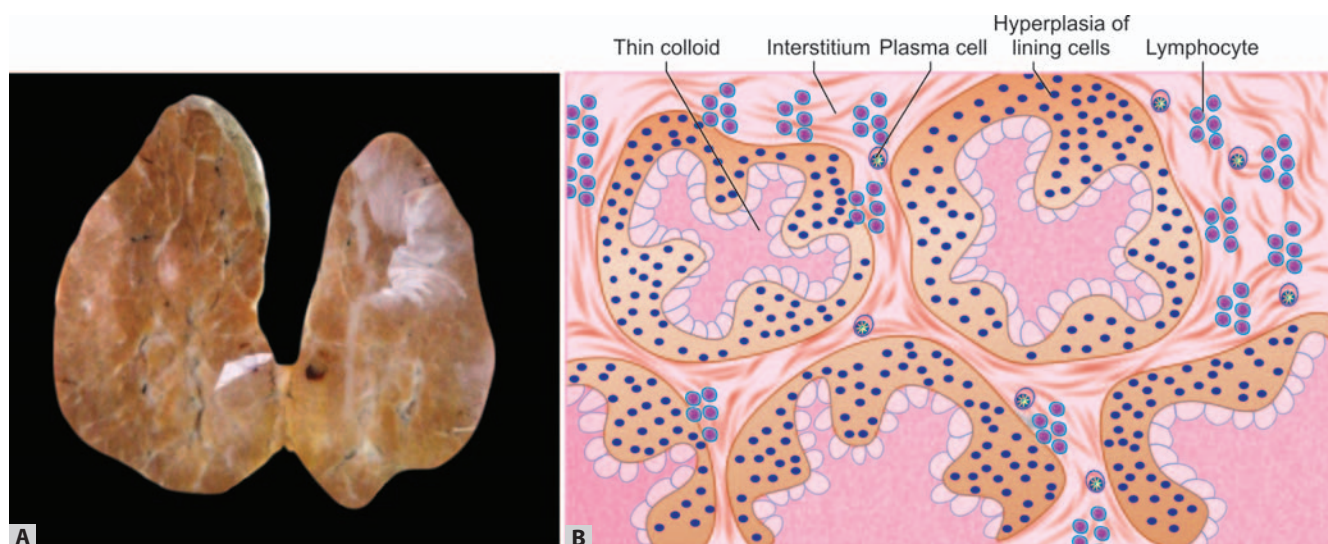
Autoimmunity is responsible for infiltrative ophthalmopathy.

MORPHOLOGY

Q. Describe the morphological changes in Graves' disease.

Gross

- Thyroid gland is **symmetrically enlarged** due to **diffuse hypertrophy and hyperplasia** of thyroid follicular epithelial cells.
- **Weight** of the gland is **increased**.
- **Cut section:** The parenchyma appears **soft and meaty** resembling normal muscle (Fig. 25.5A).



Figs 25.5A and B: Graves' disease. (A) Cut section of thyroid showing meaty appearance; (B) Microscopic view (diagrammatic) showing follicles lined by tall, columnar epithelium that are crowded and project into the lumens of the follicles. The follicle contains pale colloid with scalloped appearance of the edges. Stroma shows lymphocytes and plasma cells

Graves' disease: Thyroid is symmetrically enlarged because of diffuse hypertrophy and hyperplasia.

Microscopy (Fig. 25.5B)

A. Changes in Thyroid

- **Thyroid follicles:**
 - **Crowding of epithelial cells:** Epithelial cells lining the thyroid follicles are **tall and more crowded** than normal gland.
 - **Small papillae without fibrovascular cores:** They are formed **due to crowding of epithelial cells** may form (in contrast the papillary carcinoma has papillae with fibrovascular core).
 - The **papillae project into the lumen** of the follicles and **encroach on the colloid**.
- **Colloid:** It is **pale with scalloped margins**.
- **Lymphocyte infiltration in the interstitium:** It is seen throughout the gland, along with **mature plasma cells**. These lymphoid aggregates commonly show **germinal centers**.

B. Changes in Extrathyroidal Tissue

- Generalized lymphoid hyperplasia
- **Myocardial hypertrophy** and ischemic changes
- **Ophthalmopathy** (refer clinical features)
- **Dermopathy** (refer clinical features)

Clinical Features

Thyroid storm: Tachyarrhythmias, hyperpyrexia, shock and coma.

- **Thyrotoxicosis:** Its degree varies.
- **Unique features:**
 - **Diffuse hyperplasia of the thyroid:** It is seen in all cases and causes enlargement of thyroid.
 - **Ophthalmopathy:** The ophthalmopathy causes **abnormal protrusion of the eyeball (exophthalmos)**. Sympathetic overactivity may produce a characteristic **wide, staring gaze and lid lag**. Infiltrative ophthalmopathy is characterized by increase in the volume of the retro-orbital connective tissues and extraocular muscles.
 - **Infiltrative dermopathy or pretibial myxedema:** It is present in a minority of patients. This is most commonly seen in the skin overlying the **shins**. It presents as **scaly thickening and induration** of the dermis due to deposition of glycosaminoglycans and infiltration by lymphocytes.

Graves' disease has increased risk for other autoimmune diseases:

- Systemic lupus erythematosus
- Pernicious anemia
- Type 1 diabetes
- Addison disease.

Laboratory Findings in Graves' Disease

- **Elevated free T₄ and T₃ levels**
- **Decreased TSH levels**
- **Radioactive iodine uptake is increased, and radioiodine scans show a diffuse uptake of iodine.** This is because of continuous stimulation of the thyroid follicles by thyroid-stimulating immunoglobulins.

Radioactive iodine uptake: Evaluates synthetic capacity of thyroid.

TSH levels: Best screening test for thyroid dysfunction.

Increased radioactive iodine uptake: Increased thyroid hormone synthesis; Graves' disease.

Laboratory findings in Graves' disease: Elevated free T₄ and T₃ levels and decreased TSH levels.

DIFFUSE AND MULTINODULAR GOITERS

Q. Describe the etiopathogenesis of multinodular goiter.

Goiter: Enlargement of thyroid gland.

Definition: Goiter is defined as **enlargement of thyroid without hyperthyroidism.**

- It is the **most common manifestation of thyroid disease.**
- Two morphological forms of goiter are: (1) diffuse nontoxic goiter and (2) multinodular goiter.

Diffuse Nontoxic (Simple) Goiter

Nontoxic goiter: Absolute or relative deficiency of thyroid hormone.

- Diffuse nontoxic (simple) goiter is characterized by the **diffuse enlargement of the thyroid gland without any nodularity.**
- Microscopically, it consists of large thyroid follicles distended with colloid → also known as **colloid goiter.**

Nontoxic goiter: Hyperplasia/hypertrophy → diffuse enlargement of thyroid gland.

Etiology

Q. Write short note on goitrogens.

Types: (A) endemic and (B) sporadic.

Goiter:

- Endemic
- Sporadic.

A. Endemic Goiter

Endemic goiter: Goitrogens include vegetables of cruciferae family (cabbage, cauliflower, Brussels sprouts, turnips) and cassava.

This term is used **when goiters are present in more than 10% of the population in a given region.** The causes are:

- **Deficiency of iodine:** This may be due to **low iodine** in the soil, water, and food:
 - Common in mountainous areas (e.g. Himalayas) where there is widespread deficiency of iodine.
 - Dietary supplementation of iodine has reduced the frequency and severity of endemic goiter.
 - **Consequences of iodine deficiency (Fig. 25.6): Decreased synthesis of thyroid hormone** → causes compensatory **increase in TSH** → leads to **follicular cell hypertrophy and hyperplasia** and goitrous enlargement.
- **Goitrogens:** These are **substances ingestion of which interferes with thyroid hormone synthesis.** Goitrogenic substances include vegetables which belong to:
 - **Brassicaceae (Cruciferae) family:** For example cabbage, cauliflower, Brussels sprouts, and turnips
 - **Cassava root:** It contains a thiocyanate that inhibits iodide transport within the thyroid. Consumption of this may worsen the concurrent iodine deficiency.

B. Sporadic Goiter

- **Less frequent** than endemic goiter
- **Age:** Puberty or in young adult life
- **Sex:** Female preponderance.
- **Causes:**
 - **Hereditary enzymatic defects** that interfere with thyroid hormone synthesis. Transmitted as autosomal-recessive conditions (e.g. dysmorphonogenetic goiter).
 - **Ingestion of substances** that interfere with thyroid hormone synthesis.
 - **Unknown cause:** In most cases of sporadic goiter the cause is not known.

Sequences of events in the development of goiter are shown in Figure 25.6.

MORPHOLOGY

Q. Describe the morphological changes in multinodular goiter.

Diffuse nontoxic goiter has **two phases:**

Diffuse nontoxic goiter: Two phases

1. Hyperplastic phase
2. Phase of colloid involution.

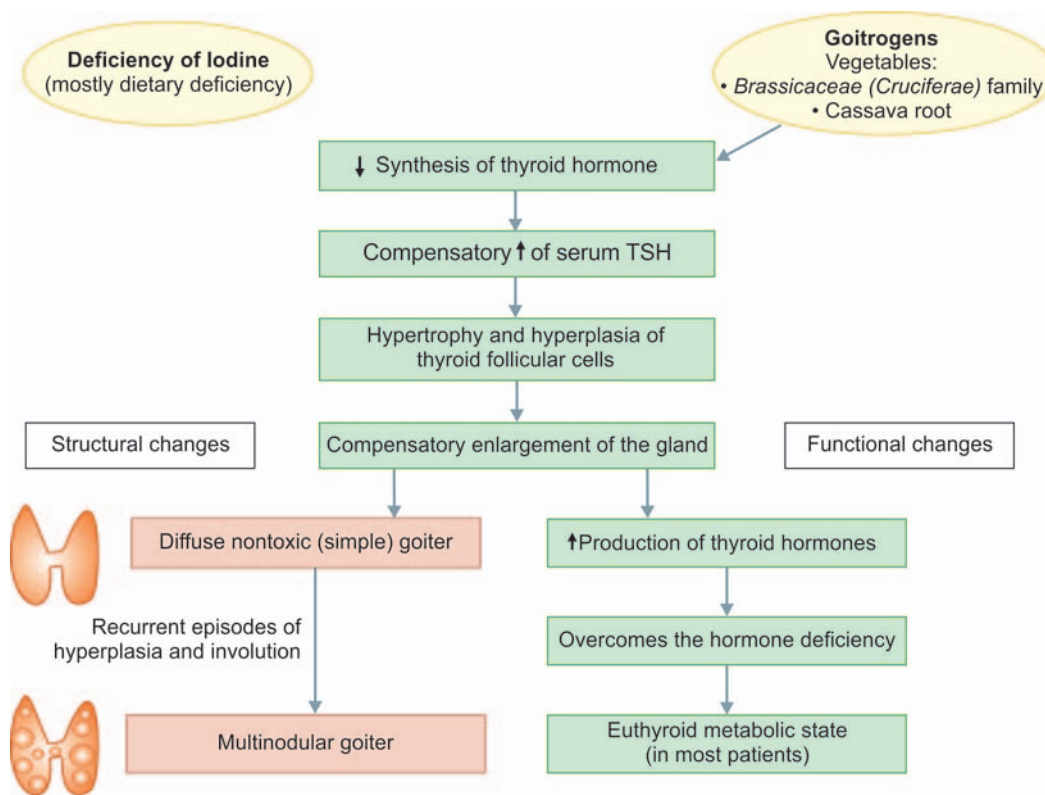


Fig. 25.6: Sequences of events in the development of goiter

1. Hyperplastic Phase:

- **Gross:** Thyroid is **moderately, diffusely, and symmetrically enlarged**. The gland rarely exceeds 100–150 grams.
- **Microscopy:**
 - **Hyperplasia of lining epithelium** of thyroid follicles. The epithelium consists of crowded columnar cells, which may pile up to form **pseudopapillae** (Sanderson's Polster) and project into the follicular lumen.
 - **Colloid content varies** throughout the gland. Some follicles are distended with colloid, whereas others are small with minimal colloid.

2. Phase of Colloid Involution

- Subsequently, if the dietary content of iodine increases or if the demand for thyroid hormone decreases, the stimulated hyperplastic phase goes into phase of colloid involution.
- **Gross:** Thyroid is **enlarged** and the **cut surface** is usually **brown, glassy, and translucent**.
- **Microscopy:**
 - **Flattened follicular epithelial lining**
 - **Abundant colloid** causes enlargement of follicle (colloid goiter).

Clinical Course

- **Majority** with simple goiters are **clinically euthyroid**
- **Mass effects** from the enlarged thyroid gland
- In children, dyshormonogenetic goiter due to congenital biosynthetic defect may produce cretinism.

Laboratory Findings

- Serum T_3 and T_4 levels are normal
- **Serum TSH is usually elevated** or at the upper range of normal.

Multinodular Goiter

Multinodular goiter: Can produce marked enlargements of thyroid and may be mistaken for neoplasm.

- In long-standing simple goiters (diffuse and symmetric enlargement), **recurrent episodes of hyperplasia and involution** combine to produce a more **irregular enlargement of the thyroid** known as multinodular goiters.
- Since, multinodular goiters are derived from simple goiter, they occur in both **sporadic and endemic** forms.

Etiology (Refer etiology of diffuse nontoxic goiter pages 719–720).

Evolution of Multinodular Goiter

- In long-standing simple goiters, due to **variations among follicular cells** in their **response to external stimuli** (such as trophic hormones) multiple nodules are formed.
- **Uneven follicular hyperplasia:**
 - Some cells in a follicle contain **clones of proliferating cells**, which may become **autonomous and proliferate** without the external stimulus → **produces new follicles**.
 - Some follicles may **accumulate colloid** without **proliferation** of epithelium → **uneven accumulation of colloid**.
- **Consequences: Produces physical stress** on both follicle and surrounding blood vessels.
 - **Rupture of follicles** may result in **colloid cysts**.
 - **Rupture of vessels** may produce **hemorrhages** followed by **scarring**, and sometimes **calcifications**. With scarring, **multiple nodules** appear and these nodules show **varying microscopic appearance**.

MORPHOLOGY

Multinodular goiter: Multiple nodules of varying sizes.

Gross (Fig. 25.7):

- **Multiple nodules:** Thyroid is **asymmetrically enlarged**, and **nodular** due to **multiple nodules of varying sizes**.
- **Pattern of enlargement:** Varies
 - **One lobe of thyroid may be more involved** than the other. It may produce pressure on midline structures, such as the trachea and esophagus.
 - **One nodule may become so prominent** and appear as a **solitary nodule**.
 - Goiter may **grow behind the sternum** and clavicles to produce **intrathoracic** or **plunging goiter**.
- **Weight of thyroid:** It is **increased** and varies.
- **Cut section:**
 - Shows **numerous irregular nodules** containing **variable amounts of colloid**.
 - **When the nodules contain large amounts of colloid**, they appear **soft, glistening, and reddish-brown**, due to gelatinous colloid.
 - **Older lesions** may show areas of **hemorrhage, fibrosis, calcification, and cystic change**.

Plunging goiter is: Retrosternal goiter.

Microscopy (Fig. 25.8)

- **Multiple nodules of varying size and shape**

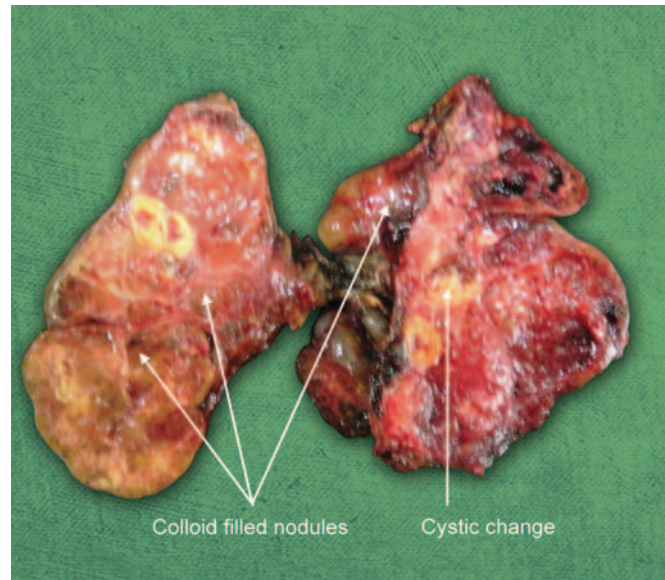


Fig. 25.7: Gross appearance of multinodular goiter showing nodular gland, containing colloid filled nodules, areas of fibrosis and cystic change

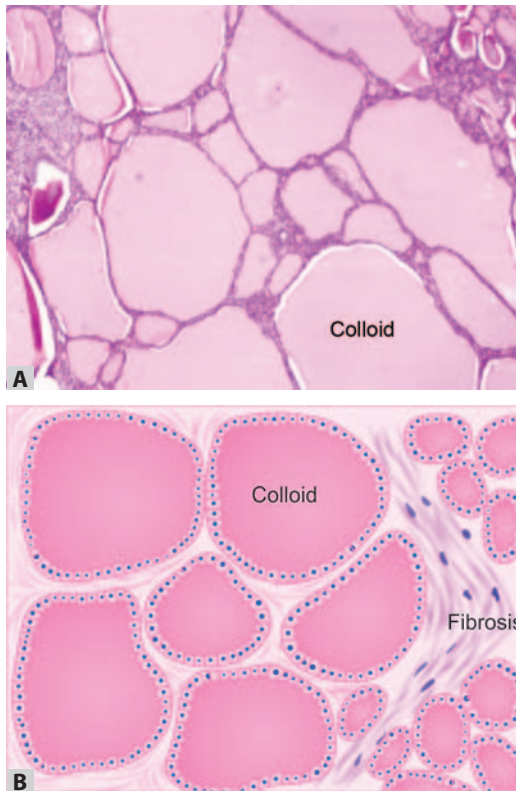
- **Nodules:** They consist of **follicles of varying sizes distended with colloid** and lined by **flat to cuboidal inactive epithelium**.
- **Colloid cysts:** They may be formed **fusion (or rupture)** of large **colloid-containing follicles**.
- **Follicular hyperplasia:** Some nodules may show follicular hyperplasia to produce **pseudopapillae** that project into the follicular lumen. These may contain either **minimal or no colloid** and **may be mistaken for follicular adenoma**. In contrast to follicular adenoma, no prominent capsule is seen between the hyperplastic nodules and residual compressed thyroid parenchyma (Table 25.1).
- **Stroma:**
 - **Fibrosis and dystrophic calcification**
 - **Areas of hemorrhage and chronic inflammation** are common. Presence of **hemosiderin deposits and cholesterol granulomas** indicate old hemorrhage.

Clinical Course

Toxic multinodular goiter: One or more nodules become TSH independent.

Exophthalmos and pretibial myxedema are NOT seen in toxic multinodular goiter.

- **Usually asymptomatic** and present as a **mass in the neck**.
- **Large multinodular goiter** may cause **compression of surrounding structures** and may cause:
 - **Airway obstruction** due to compression of trachea.
 - **Dysphagia** by compressing the esophagus.



Figs 25.8A and B: (A) Photomicrograph; and (B) Diagrammatic. Microscopy of a multinodular goiter composed of thyroid follicles of varying sizes distended with variable amount of colloid

- **Venous congestion of the head and face** (superior vena cava syndrome) due to compression of large vessels in the neck and upper thorax.
- **Hoarseness** from recurrent laryngeal nerve compression.
- **Functional status:**
 - **Most patients are euthyroid** and T_4 , T_3 , and TSH are normal.
 - Some may have subclinical hyperthyroidism with reduced TSH levels.
 - Few may develop hyperthyroidism (toxic multinodular goiter) and this condition is known as **Plummer syndrome**.
- Fine-needle aspiration biopsy is helpful for diagnosis.

Plummer syndrome

- Multinodular goiter
- Hyperthyroidism.

NEOPLASMS OF THE THYROID

Tumors of the thyroid are usually benign clinical criteria that generally provide clues to the nature of a thyroid nodule (Table 25.1).

TABLE 25.1: Clinical criteria that generally provide clues to the nature of a thyroid nodule

More likely to be neoplastic	More likely to be benign
<ul style="list-style-type: none"> • <i>Solitary nodules</i> than multiple nodules • <i>Nodules in younger patients</i> than are those in older patients • <i>Nodules in males</i> than in females • A history of <i>radiation</i> treatment to the head and neck region 	<ul style="list-style-type: none"> • Functional nodules that take up radioactive iodine in imaging studies (<i>hot nodules</i>)

Thyroid neoplasms: Majority present as solitary thyroid nodules, but only ~1% of all thyroid nodules is neoplastic.

Follicular Adenomas

Q. Write short note on follicular adenoma of thyroid.

Adenomas of the thyroid consist of follicular epithelium.

Follicular adenoms: Most common benign tumor of thyroid.

Pathogenesis

Nonfunctional Follicular Adenoma

- **Majority** of adenomas **does not produce thyroid hormones**.
- **Genetic factors:** **Less than 20%** of nonfunctioning follicular adenomas patients have anyone of the following genetic alterations:
 - **Mutations of RAS** (signal transduction protein)
 - **Phosphatidylinositol-3-kinase subunit (PIK3CA)**
 - **PAX8-PPARG** fusion gene.

All these are genetic alterations also seen in follicular carcinomas.

Functional Follicular Adenoma

- **They produce thyroid hormones (toxic adenomas)** and cause **thyrotoxicosis**.
- **Somatic mutations of TSH receptor signaling pathway:** It has been found in toxic adenomas and in toxic multinodular goiter → leads **secretion of thyroid hormone** by follicular cells independent of TSH stimulation (thyroid autonomy).

MORPHOLOGY

Gross (Fig. 25.9)

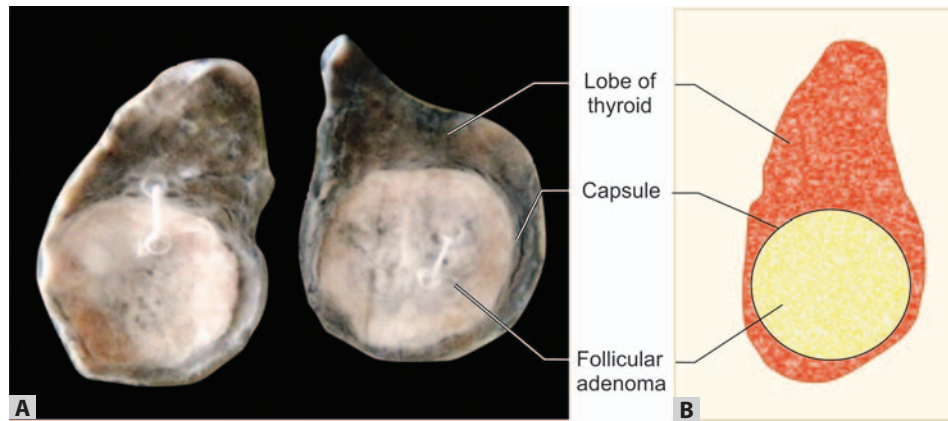
- Follicular adenoma is a **solitary** (single), **spherical, solid** and **encapsulated** tumor.

- **Size:** It ranges from 1–3 cm in diameter.
- **Cut surface:**
 - Tumor is **soft and paler** than the surrounding gland
 - **Well-demarcated** and surrounded by a **thin intact, well-formed fibrous capsule**
 - Tumor **compresses the adjacent thyroid gland**
 - **Cut surface of tumor bulges** when fresh
 - **Color** may range from **gray-white to red-brown**.
- **Secondary changes:** They are common and include **hemorrhage, fibrosis, calcification, and cystic change**.
Follicular adenoma should be differentiated from solitary dominant nodule of a multinodular goiter (Table 25.1).

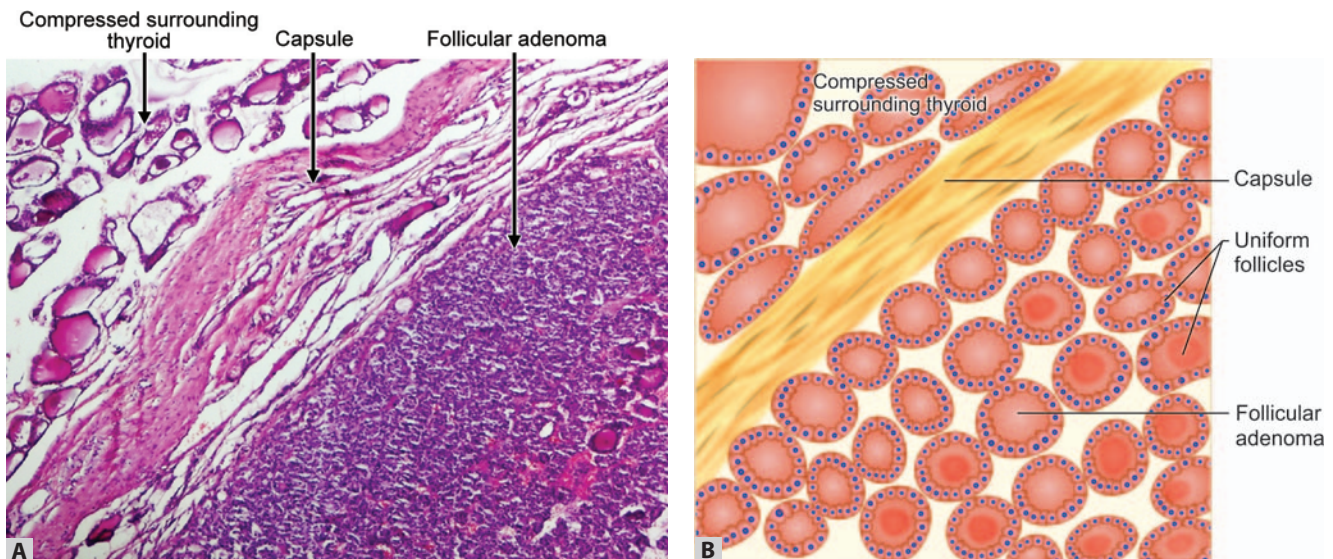
Microscopy (Fig. 25.10):

Patterns: Follicular adenomas may show **many histologic patterns**, which **do not have of any significance**. The tumor cells are arranged in follicles, which may **resemble normal thyroid tissue or mimic different stages in the embryonic development of the gland**.

- **Embryonal (trabecular) adenoma:** It consists of follicular cells arranged in a **trabecular pattern** in which poorly formed follicles contain little or no colloid.
- **Fetal (microfollicular) adenoma:** It consists of tumor cells which are similar to those of embryonal adenoma, but tend to be arranged in **microfollicles** (small follicles) containing **little colloid**.



Figs 25.9A and B: Gross (A) specimen and (B) diagrammatic appearance of a follicular adenoma



Figs 25.10A and B: (A) Photomicrograph; and (B) Diagrammatic: Microscopic appearance of part of follicular adenoma of the thyroid showing well-differentiated, uniform thyroid follicles, well-formed capsule and compressed adjacent thyroid follicles

Q. Differences between adenoma and goiter.

TABLE 25.2: Comparison of adenomatous goiter nodule and follicular adenoma

Characteristics	Nodule of a nodular goiter	Follicular adenoma
Gross		
Number of nodules	Multiple	Single
Encapsulation	Poor	Good
Microscopy		
Structure of follicles within the nodule	Variable	Uniform
Growth pattern in adjacent gland	Comparable growth pattern in adjacent gland	Distinct architecture inside and outside the capsule
Compression of adjacent gland	No compression	Seen outside the capsule

- **Simple adenoma:** It shows **mature follicles** with a **normal amount of colloid**.
- **Colloid (macrofollicular) adenoma:** It is similar to simple adenoma except that the **follicles are larger** and contain **more abundant colloid**.
- **Hürthle cell adenoma:** It is a solid tumor **composed of oxyphil cells**, small follicles, and scanty colloid.
- **Atypical adenoma:** It is a follicular tumor with **mitoses, excessive cellularity, nuclear atypia** or equivocal capsular invasion.

Differences between nodular goiter from follicular adenoma are shown in Table 25.2.

Clinical Features

- Mostly present as a **unilateral painless mass**
- Larger tumors may produce local symptoms.

Solitary thyroid nodule in female: Majority are benign.

Investigations

- **Radionuclide scanning:**
 - **Nonfunctioning adenomas:** They take up less **radioactive iodine** than does normal thyroid parenchyma. The nonfunctioning adenomas usually appear as **cold nodules**. About 10% of cold nodules may be malignant on microscopic examination.
 - **Functioning follicular adenoma** (toxic adenomas): They appear as **hot nodules**. **Malignancy is rare** in hot nodules.
- **Ultrasonography.**
- **Fine-needle aspiration biopsy.**
- **Histological examination** of surgically resected specimen should be evaluated for capsular integrity, and definitive diagnosis of adenomas.

Prognosis: Excellent and adenomas do not recur or metastasize.

Solitary thyroid nodule in child/male: More likely malignant.

CARCINOMAS

- Carcinomas of the thyroid are **relatively uncommon**.
- **Sex:**
 - **Early and middle adult years:** **Female** predominance.
 - **Childhood and late adult life:** Males and females **equally affected**.
- **Nature:** **Majority** of thyroid carcinomas are **well-differentiated**.

Major Subtypes

Papillary carcinoma: Most common malignant tumor of thyroid.

1. Papillary carcinoma (more than 85%)
2. Follicular carcinoma (5–15%)
3. Anaplastic (undifferentiated) carcinoma (less than 5%)
4. Medullary carcinoma (5%).

Papillary carcinoma: Most common endocrine malignancy.

Pathogenesis of Thyroid Carcinomas

Origin

Follicular cell-derived thyroid carcinoma:

1. Follicular carcinoma
2. Papillary carcinoma
3. Anaplastic carcinoma.

- **Follicular cell-derived malignancies:** These include three major types of thyroid cancers namely (1) **follicular**, (2) **papillary** and (3) **anaplastic carcinoma**.
- **Non-follicular cancer:** One type namely **medullary carcinomas** do not arise from the follicular epithelium.

Two factors play major role in thyroid carcinoma: (A) genetic factors and (B) environmental factors.

Thyroid carcinoma: Different genetic changes are involved in its pathogenesis.

A. Genetic Factors (Fig. 25.11)

Different genetic changes are involved in the pathogenesis of the four major histologic variants of thyroid cancer.

Follicular Cell-derived Malignancies

Follicular cell-derived thyroid carcinoma: Mutations in two signaling pathways namely:

1. Mitogen-activated protein (MAP) kinase pathway
2. Phosphatidylinositol-3-kinase (PI-3K)/AKT pathway.

General features: Genetic alterations in the three follicular cell-derived malignancies results in **gain-of-function mutations** along components of the **two signaling pathways** namely:

- **Mitogen-activated protein (MAP) kinase pathway**
- **Phosphatidylinositol-3-kinase (PI-3K)/AKT pathway**
 - Normally, these two signaling pathways are transiently activated by binding of soluble growth factor ligands to the extracellular domain of receptor tyrosine kinases.

This binding leads to autophosphorylation of the cytoplasmic domain of the receptor, and results in intracellular signal transduction.

- **Mutation** involving the genes involved in the above two pathways → **leads to oncogenic constitutive activation** even in the absence of ligand → promote **carcinogenesis**.

1. **Papillary carcinomas: MAP kinase pathway** activation is a major feature of most papillary carcinomas. The activation can occur by one of the two major mechanisms.

Papillary carcinoma: Chromosomal rearrangements involving RET/PTC fusion gene and BRAF mutations.

- **Activation of growth factor receptor:** It is the first method of activation of MAP kinase pathway. Two genes encode transmembrane tyrosine kinase growth factor receptors namely: (1) *RET* and (2) *NTRK1* (neurotrophic tyrosine kinase receptor).
 - **RET gene:** It is **normally not expressed in thyroid follicular cells**. In papillary cancers, chromosomal (somatic) rearrangements of the *RET* gene are common. These rearrangements **cause the fusion of the tyrosine kinase domain of RET to various other genes** → creates **RET/PTC (RET/papillary thyroid carcinoma) fusion oncogenes** → product is RET/

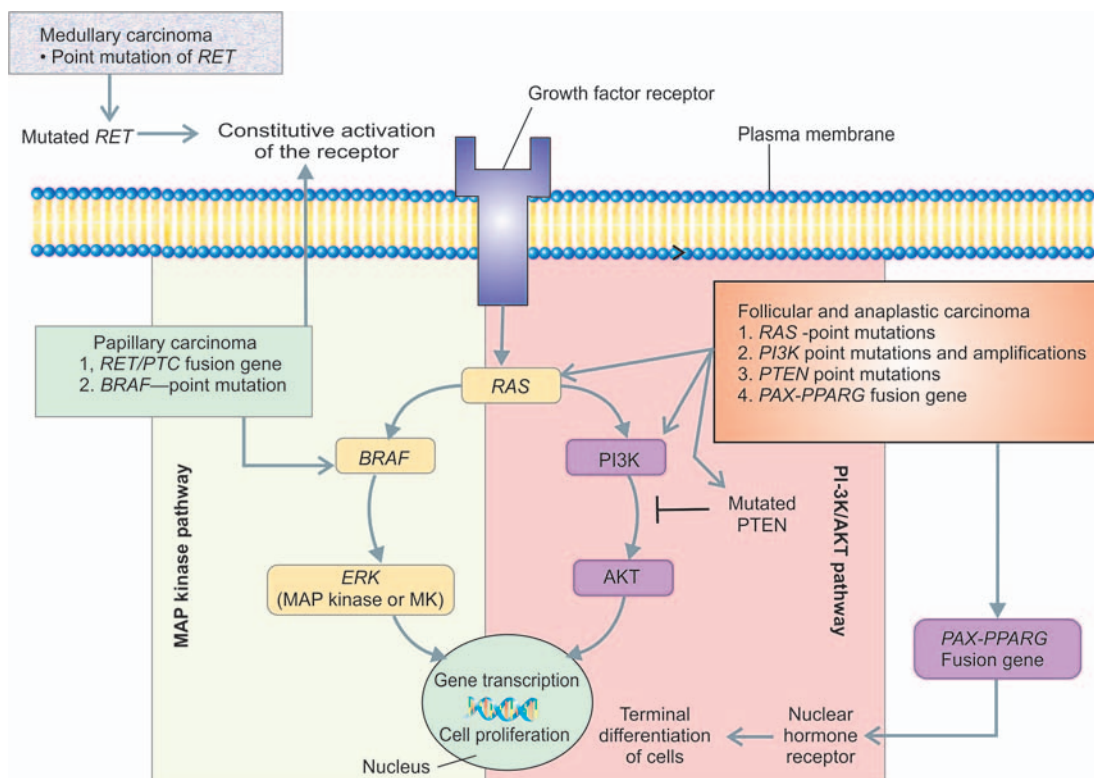


Fig. 25.11: Genetic alterations in follicular cell-derived malignant tumors and medullary carcinoma of the thyroid gland

PTC fusion protein → constitutive activation of the tyrosine kinase receptor in thyroid follicular cells → activation of the MAP kinase pathway.

- ♦ **RET/PTC:** It is present in about 20–40% of papillary thyroid cancers and frequency is higher those following radiation exposure.
- **Neurotrophic tyrosine kinase receptor 1 (NTRK1) gene:** Its fusion with another gene → resultant fusion proteins → constitutively expressed in thyroid cells → leads to activation of MAP kinase.
- ♦ It observed in 5–10% of papillary thyroid cancers.

RET gene codes for growth factor tyrosine kinase receptors.

RET/PTC fusion protein: Causes constitutive activation of the tyrosine kinase growth factor receptor in thyroid follicular cells.

- **Mutations in signal transduction genes:** It is the second method of activation of MAP kinase pathway is by **activating point mutations in BRAF**, whose product is an intermediate signaling component in the MAP kinase pathway. BRAF is a RAS signal transduction protein.
- **Point mutation of BRAF gene:** It is observed in ~30–50% of papillary carcinomas and is **associated with metastatic disease and extrathyroidal extension**.

RET/PTC rearrangements and BRAF point mutations are **not found in follicular adenomas or carcinomas**.

BRAF gene codes for RAS signal transduction protein.

Papillary carcinoma:

1. RET/PTC fusion gene
2. BRAF mutation.

2. Follicular carcinomas

Follicular carcinoma: Point mutations of:

- RAS
- PI3K
- PTEN

PAX8/PPARG fusion gene.

- a. **Mutations in PI-3K/AKT cell signaling pathway:** It is observed in ~30–50% of follicular carcinomas. This mutations result in constitutive activation of this oncogenic pathway. The mutations may involve different subset of PI-3K/AKT signaling. These includes:
 - Tumors with gain-of-function point mutations of **RAS** (signal transduction protein) and **PIK3CA**
 - Tumors with amplification of **PIK3CA**
 - **PTEN** is a **tumor suppressor gene** and negative regulator of this pathway. Follicular carcinoma may show **mutations of PTEN with loss-of-function of this tumor suppressor gene**.

PTEN is a tumor suppressor gene.

- **PAX8-PPARG fusion gene:** A unique (2;3)(q13;p25) translocation was found in 30–50% of follicular carcinomas → produces a fusion gene composed of portions of **PAX8** (gene involved in thyroid development), and the peroxisome proliferator-activated receptor gene (**PPARG**), and its product is a nuclear hormone receptors implicated in terminal differentiation of cells.
- 3. **Anaplastic (undifferentiated) carcinomas:** These are highly **aggressive and lethal tumors**. They can arise de novo or more commonly by “dedifferentiation” of a well-differentiated follicular or papillary carcinoma.
 - Molecular changes are similar those found in well-differentiated carcinomas (e.g. **RAS** or **PIK3CA** mutations), but occur at a higher rate.
 - **Other genetic changes:** Inactivation of **p53** or mutations of **β-catenin**, are seen in anaplastic carcinomas and are associated with aggressive behavior.

Medullary Thyroid Carcinomas

Medullary carcinoma: Point mutation of RET gene.

- **Genes coding growth factor receptors: Familial medullary thyroid carcinomas** occur in multiple endocrine neoplasia type 2 (MEN-2) and are associated with **germline RET gene mutations**. The **RET gene codes for receptor tyrosine kinase and oncogenic version of RET gene** (mutated RET) lead to **constitutive activation of the tyrosine kinase receptor and cell proliferation**.
- RET mutations are also seen in about 50% of nonfamilial (sporadic) medullary thyroid cancers.
- **RET/PTC** observed in papillary carcinomas is **not seen in medullary carcinomas**.

B. Environmental Factors

1. **Ionizing radiation:** It is the major risk factor mainly during the first 2 decades of life.
2. **Deficiency of dietary iodine** and associate goiter may be associated with a higher frequency of follicular carcinomas.

Papillary Carcinoma

Q. Write short note on papillary carcinoma of thyroid.

Papillary carcinoma:

- Accounts for 85% of thyroid malignancy in iodine-deficient areas
- Most common thyroid cancer in children and in persons exposed to external radiation
- Most often in women between 30–40 years
- Excellent prognosis.

- The **most common** and constitutes ~ 85% of primary thyroid cancer.
- **Age:** It can occur at any age, but **mostly** found between the ages of **25 and 50**.
- Associated with **previous exposure** to **ionizing radiation**.
- Genetic factors (refer under pathogenesis of thyroid carcinoma).

MORPHOLOGY

Gross

- Papillary carcinomas may be **solitary or multifocal** lesions.
- Tumors may be: (1) **well-circumscribed**, (2) **encapsulated** or (3) **ill-defined** (infiltrate the adjacent parenchyma).
- **Gray white, firm to hard tumor**, which may show areas of fibrosis and calcification. May also be cystic.
- **Cut surface** may show **papillary foci**.

Microscopy (Fig. 25.12)

Characteristic nuclear features:

1. Ground glass (Orphan Annie) nuclei
2. Pseudoinclusions
3. Nuclear grooves.

- **Complex branching papillae:** They have a dense central fibrovascular stalk/core.
 - Papillae are covered by a **single to multiple layers of uniform cuboidal to columnar epithelial cells**.

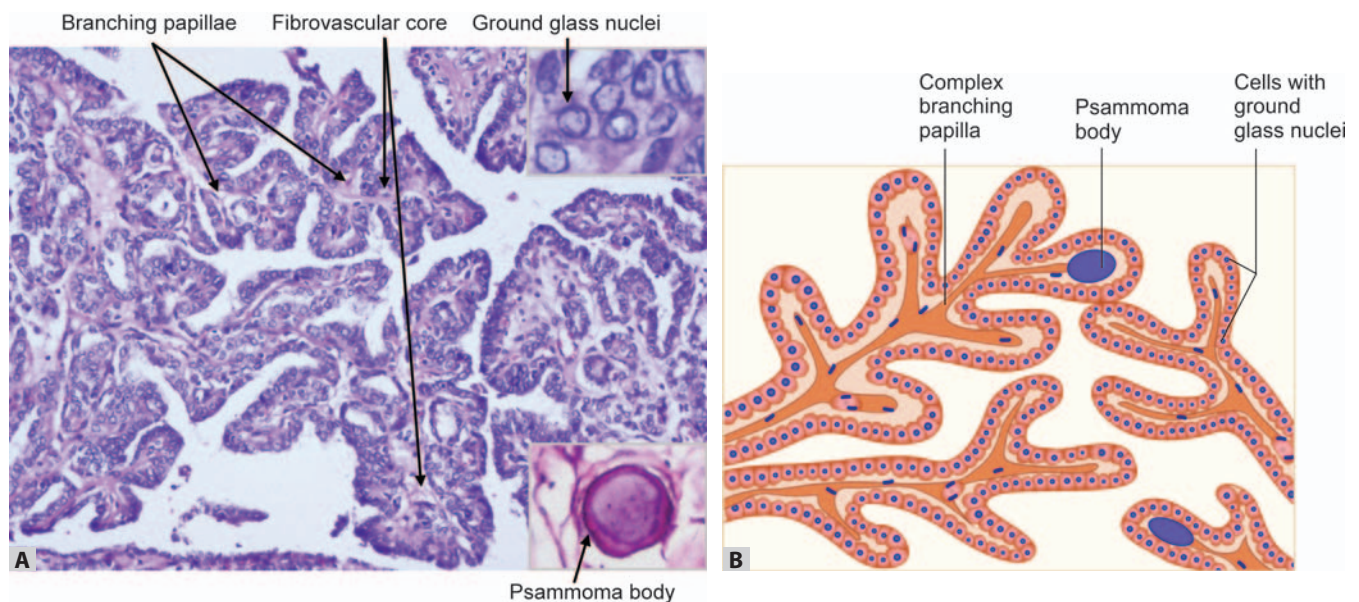
- **Nuclear features:** These are **important for diagnosis** of papillary carcinoma, **even in the absence of papillary architecture**.
 - **Ground glass or Orphan Annie eye nuclei:** They contain **finely dispersed chromatin**, which gives an **optically clear or empty** appearance.
 - **Intranuclear inclusions and intranuclear grooves:** Invaginations of the cytoplasm into the nucleus in cross-sections may give the appearance of eosinophilic **intranuclear inclusions** (pseudoinclusions) or **intranuclear grooves**.
- **Psammoma bodies** (calcospherites): These are **concentrically calcified structures usually present within the papillary core**. They are virtually **diagnostic** of papillary carcinoma, and almost **never found in follicular and medullary carcinomas**.
- **Lymphatic spread:** Lymphatic invasion and spread to regional cervical lymph nodes are **common**, but **vascular invasion and blood spread are uncommon**.

Papillary carcinoma:

- Nuclear features are used as diagnostic criteria even in the absence of papillae.

Histological Variants of Papillary Carcinoma

- **Follicular variant:** It is the **most common variant**, in which the **nuclei show characteristic features of papillary carcinoma** but has totally **follicular architecture**.
- **Tall-cell variant:** It is characterized by **tall columnar cells with intensely eosinophilic cytoplasm** lining the papillary structures.



Figs 25.12A and B: (A) Photomicrograph; (B) Diagrammatic. Papillary carcinoma of the thyroid shows well-formed, branching papillae lined by cells with characteristic empty-appearing ("Orphan Annie eye") nuclei. Inset in A: upper right shows Orphan Annie eye nuclei and nuclear groove and right lower shows psammoma body

- **Diffuse sclerosing variant:** It shows prominent papillary projections, admixed with nests of squamous metaplasia, **extensive, diffuse fibrosis** with a **prominent lymphocytic infiltrate**.
- **Papillary microcarcinoma:** It is **less than 1 cm** in size, and usually found as an **incidental finding on surgery**.

Clinical Course

Papillary carcinoma: Most commonly spreads through lymphatics.

Most papillary carcinomas present as **asymptomatic thyroid nodules**, sometimes the presenting symptom may be a mass in a cervical lymph node (due to metastasis).

- **Fine-needle aspiration cytology** shows nuclear features characteristic of papillary carcinoma.
- **Prognosis:** Excellent.

Papillary carcinoma: Metastasize through lymphatics and have excellent prognosis.

Follicular Carcinoma

Follicular carcinoma: Most common thyroid cancer presenting as a solitary cold nodule.

- Follicular carcinomas constitute ~ 5–15% of primary thyroid cancers.
- Sex: More common in **women** (3:1).
- Age: Develop at an **older age than papillary carcinoma** with a peak incidence between **40 and 60 years** of age.

Etiology

- More **frequent in regions** where there is **dietary deficiency of iodine**. Genetic factors (refer under pathogenesis of thyroid carcinoma).

MORPHOLOGY

Gross

- Follicular carcinomas are **single nodules** which may be **either well-circumscribed or infiltrate** into the surrounding thyroid parenchyma.
 - Circumscribed tumors may be difficult to distinguish from follicular adenomas on gross examination.
 - Large tumors may penetrate the capsule and infiltrate into the adjacent structures in the neck.
- Cut section of tumor appears **gray to pink** in color.

Follicular thyroid carcinoma is differentiated from follicular adenoma:

- Capsular invasion
- Vascular invasion.

Microscopy

- **Follicular pattern of tumor cells:**
 - Tumor consists of **uniform cuboidal to columnar** follicular epithelial cells.
 - Tumor cells may form **small follicles containing colloid** or may be arranged in **nests or sheets** without forming follicles.
 - **Increased mitotic activity**.
 - Neither nuclear feature of papillary carcinoma nor psammoma bodies is seen.
- **Invasion: Depending on the pattern of invasion**, follicular carcinoma can be **subdivided into two variants**.

Follicular carcinoma:

1. Minimally invasive
2. Widely invasive.

1. Minimally invasive follicular carcinomas:

- Gross: Tumors appear as a **well-defined and encapsulated lesion**.
- Microscopy: **Resemble follicular adenoma** but shows **mitoses, capsular and/or vascular invasion**.

2. Widely invasive follicular carcinomas: They infiltrate the thyroid parenchyma, its capsule and into the surrounding extrathyroidal soft tissues.

• Metastasis:

- **Lymphatic spread is uncommon**.
- **Hematogenous spread is common to bone, lungs and liver**.

Clinical course: Follicular carcinomas present as **slowly enlarging painless nodules**.

Follicular neoplasm: Adenoma and carcinoma. Both are composed of well-differentiated follicular epithelial cells. Follicular carcinoma shows capsular and/or vascular invasion.

Follicular carcinoma: Most commonly spreads through hematogenous than through lymphatics.

Pulsating secondaries:

1. Follicular carcinoma of thyroid
2. Renal cell carcinoma.

Anaplastic (Undifferentiated) Carcinoma

Anaplastic carcinomas: Undifferentiated tumors of follicular epithelium and are thought to arise by dedifferentiation of more differentiated tumors.

- Anaplastic carcinomas are **undifferentiated tumors** of follicular epithelium.
- Constitute less than 5% of thyroid tumors.

- **Age: Elderly** with a mean age of 65 years.
- Genetic factors (refer under pathogenesis of thyroid carcinoma).

MORPHOLOGY

Gross: Diffusely infiltrative tumor.

Microscopy: Composed of highly anaplastic cells, which includes:

1. **Spindle** cells with a sarcomatous appearance.
2. Large, pleomorphic **giant** cells.
3. **Mixed** spindle and giant cells

Immunohistochemistry: The tumor cells express epithelial markers like **cytokeratin**, but are usually **negative for thyroglobulin**.

Spread

1. **Local spread** into thyroid capsule and adjacent neck structures.
2. **Hematogenous spread** to lungs.

Clinical Course

- Usually, present as a **rapidly growing bulky neck mass**.
- Symptoms due to **compression and invasion of the neck structures** may cause dyspnea, dysphagia, hoarseness, and cough.

Prognosis: Aggressive tumor with poor prognosis.

Anaplastic carcinomas:

1. Highly aggressive
2. Poor prognosis.

Medullary Carcinoma

Q. Write short note on medullary carcinoma of thyroid.

- Medullary carcinomas of the thyroid are **neuroendocrine tumors derived from the parafollicular cells or C-cells** of the thyroid.
- Similar to normal C-cells, they **secrete calcitonin**, measurement of which is **useful in the diagnosis and postoperative follow-up**.
- It constitutes ~ 5% of thyroid neoplasms.
- Genetic factors (refer under pathogenesis of thyroid carcinoma).

Medullary carcinoma: Arises from parafollicular C-cells.

Types

Medullary carcinoma

- Sporadic (~70%)
- Familial (~30%).
- **Sporadic:** Constitutes about 70% of tumor with a peak incidence in the **40s and 50s**.

- **Familial:** It may occur:
 - **With associated MEN syndrome 2A or 2B** in younger patients.
 - **Without an associated MEN syndrome.**

Point mutations in the RET gene is seen in both familial and sporadic medullary carcinomas (Fig. 25.11).

Medullary carcinoma-familial:

- Multicentric
- C-cell hyperplasia.

Medullary carcinoma of thyroid is associated with mutation in: **RET gene**.

MORPHOLOGY

Gross

- **Number:**
 - **Solitary nodule** in **sporadic** medullary thyroid carcinomas
 - **Multicentric and bilateral common** in **familial cases**.
- Tumor is **firm, pale gray, and infiltrative**.
- **Larger lesions** may show areas of **necrosis and hemorrhage** and they **may extend through the capsule of the thyroid**.

Microscopy (Fig. 25.13)

Medullary carcinoma: Amyloid stroma is a characteristic microscopic finding.

- **Tumor cells:**
 - **Polygonal to spindle-shaped cells**. **Small, more anaplastic cells** may be found in **some** tumors.
 - Cells are arranged as **nests, trabeculae** and even form follicles.
- Acellular **amyloid deposits in the stroma** (refer Fig. 6.27): It is found in most of the cases. These are **derived from altered calcitonin polypeptides**.
- **C-cell hyperplasia:** It is seen in the **surrounding thyroid of familial medullary cancers**, which is **not observed in sporadic lesions**. They **represent the precursor lesions** for medullary carcinoma.

Immunohistochemistry: **Calcitonin** can be demonstrated within the cytoplasm of the tumor cells and in the stromal amyloid by immunohistochemical methods.

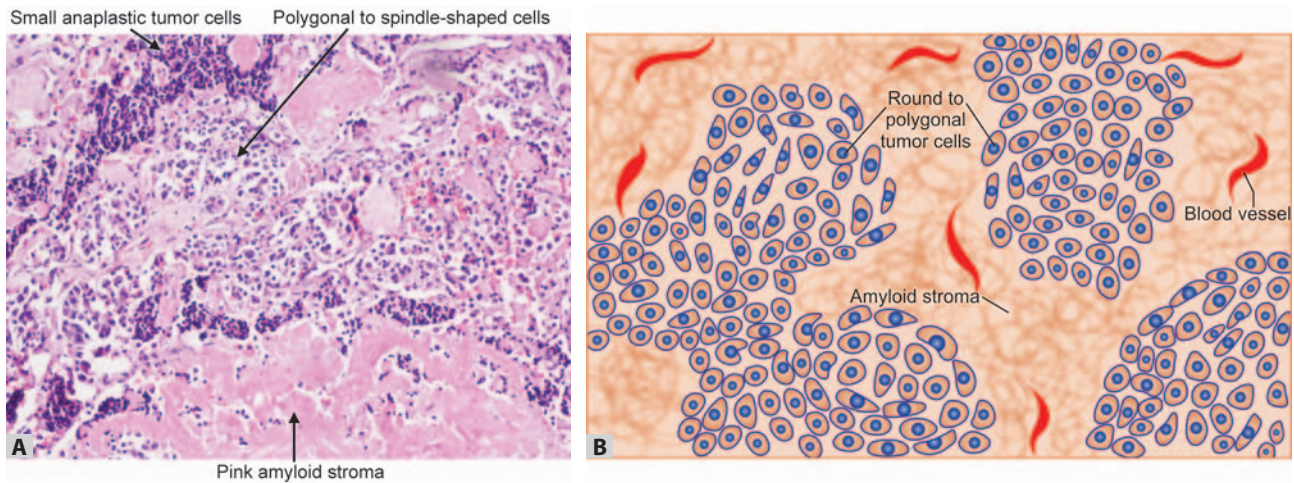
Electron microscopy: It shows **membrane-bound electron-dense granules** within the cytoplasm of the tumor cells.

Clinical Course

1. Sporadic cases:

Medullary carcinoma: Paraneoplastic syndrome in few

- Diarrhea due to vasoactive intestinal peptide (VIP)
- Cushing syndrome due to adrenocorticotrophic hormone (ACTH).



Figs 25.13A and B: (A) Photomicrography; and (B) Diagrammatic. Medullary carcinoma of thyroid shows nests and trabeculae of round, spindle to polygonal cells separated by abundant homogeneous, extracellular, pink deposits of amyloid

- Present as a **mass in the neck**
- **Paraneoplastic syndrome:** It may occur in some medullary carcinoma (e.g. diarrhea due to the secretion of VIP, or Cushing syndrome due to ACTH).
- **Tumor markers:** They are useful, mainly for presurgical assessment of tumor load and in calcitonin-negative tumors. These include:
 - Calcitonin
 - Carcinoembryonic antigen (CEA).

2. Familial cases:

Medullary carcinoma: Tumor markers

1. Calcitonin (It is converted to amyloid)
2. Carcinoembryonic antigen.

- Present with **symptoms localized to the thyroid** or as a component of familial syndromes along with neoplasms in other organs (e.g. adrenal or parathyroid glands).
- Medullary carcinomas arising as a component of MEN-2B are more aggressive and more frequently metastasize than sporadic tumors (MEN-2A, or FMTC).

NEUROBLASTIC TUMORS

Neuroblastic tumors are group of tumors of the sympathetic ganglia and adrenal medulla that are derived from primordial neural crest cells.

Neuroblastoma

Q. Write short note on neuroblastoma.

- The most **important neuroblastic tumor**
- **Age: Most common extracranial childhood solid tumor.** It is the most frequently diagnosed during infancy.

Neuroblastoma: Most common extracranial childhood solid tumor.

• Sporadic and familial types:

- Mostly occur sporadically, but 1–2% is familial.
- **Germline mutations in the anaplastic lymphoma kinase (ALK) gene** are observed in familial neuroblastoma.
- Somatic gain-of-function *ALK* mutations are also found in a few sporadic neuroblastomas.
- Tumors having *ALK* mutations respond to drugs that target their activity.

Neuroblastoma:

1. Most common malignant abdominal tumor in children
2. Most common malignant tumor of infancy.

Neuroblastoma: Malignant tumor of postganglionic sympathetic neurons arises in adrenal medulla.

MORPHOLOGY

Gross

- **Site:** About 40% of neuroblastomas occur in the **adrenal medulla**.
 - **Other sites:** It may develop along the sympathetic chain.
 - ♦ **Paravertebral region of the abdomen** (25%).
 - ♦ **Posterior mediastinum** (15%).
 - ♦ Pelvis, the neck, and brain (**cerebral neuroblastomas**).
- **Size:** **Vary** from minute nodules (as in situ lesions) to large tumors weighing 1 kg.
- **Nature:** **Majority** are **silent** and **regress spontaneously**.
- May be **sharply demarcated** by a fibrous pseudocapsule or **infiltrate** the surrounding structures (kidneys, renal vein, and vena cava, and aorta).
- **Cut section:** **Soft, and gray-tan.** Large tumors may show areas of **necrosis, cystic change** and **hemorrhage**.

Microscopy (Fig. 25.14)**Neuroblastoma:**

- Small, primitive cells with dark nuclei
- Homer-Wright pseudorosettes.
- **Tumor cells:** They are arranged in solid sheets. The tumor cells appear as:
 - **Small, primitive containing dark nuclei**
 - **Scant cytoplasm with poorly defined cell borders.**
- Mitotic activity, karyorrhexis (breakdown of nuclear material), and pleomorphism may be prominent.
- These tumors may be **difficult to differentiate morphologically from other small round blue cell tumors.**
- **Background:** Shows a faintly **eosinophilic fibrillary material (neuropil)**, which represents the neuritic processes of the primitive neuroblasts.
- **Homer-Wright pseudorosettes:** It consist of tumor cells concentrically arranged about a central space filled with neuropil may be seen.

Immunohistochemistry: Neuron-specific enolase positive.

Electron microscopy: It shows **small, membrane-bound, catecholamine-containing secretory granules in the cytoplasm.**

Maturation: Some neoplasms may show **spontaneous or induced (by therapy) maturation.** These differentiated lesions include ganglioneuroblastoma and ganglioneuroma. Maturation is **characterized by the presence of**

Schwannian stroma composed of organized fascicles of neuritic processes, mature Schwann cells, and fibroblasts. This type of stroma is required for the designation of ganglioneuroblastoma and ganglioneuroma. The presence of ganglion cells is not a criteria for maturation.

- **Ganglioneuroblastoma:** It consists of **primitive neuroblasts** may be **admixed with ganglion cells** in various stages of maturation. The ganglion cells appear as large cells with abundant cytoplasm having large vesicular nuclei and a prominent nucleolus.
- **Ganglioneuroma** (Fig. 25.15): It is a **more mature tumor than ganglioneuroblastoma.** It contains many **more large cells resembling mature ganglion cells** with few if any residual neuroblasts. Maturation of neuroblasts into ganglion cells is usually accompanied by the appearance of **Schwann cells.** The presence of **Schwannian stroma** is associated with a **favorable outcome.**

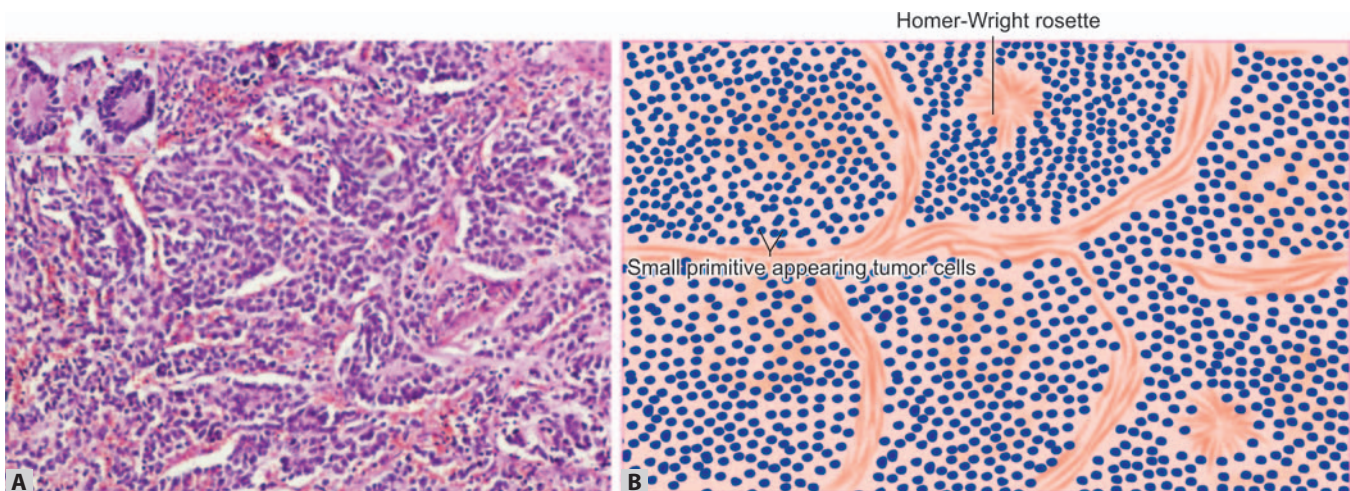
Spread of Tumor

- Local infiltration
- Lymph node spread
- Bloodspread: Liver, lungs, bone marrow, and bones.

Clinical Course

Neuroblastoma: Child with large abdominal mass.

Spontaneous regression of tumor is seen in: Neuroblastoma.



Most common cancer of childhood: Leukemia (30%) > Brain tumor (22%).

Most common solid tumor of childhood: Brain tumor.

Most common soft tissue tumor in infants and children: Rhabdomyosarcoma.

Figs 25.14A and B: (A) Photomicrograph; and (B) Diagrammatic. Neuroblastoma consists of small primitive appearing cells with scant cytoplasm embedded in a finely fibrillar matrix. Inset of A shows two Homer-Wright rosettes

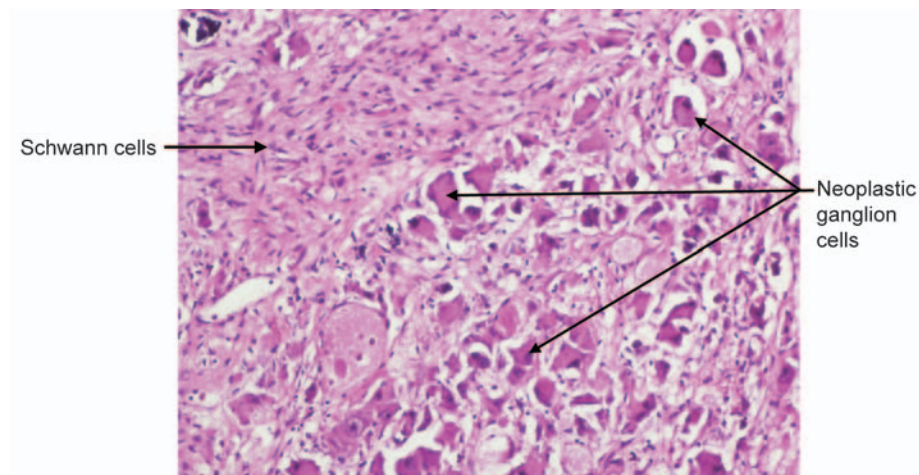


Fig. 25.15: Ganglioneuroma with numerous large cells with vesicular nuclei and abundant eosinophilic cytoplasm, representing neoplastic ganglion cells (right half). Spindle-shaped Schwann cells are present in the background stroma

- Children below 2 years of age: Usually present as **large abdominal masses, fever, and weight loss**.
 - Older children: Symptoms develop due to metastases such as bone pain, respiratory symptoms, or gastrointestinal complaints.
 - Ganglioneuromas may present either as asymptomatic mass or symptoms related to compression.
3. **Amplification of the *N-MYC* oncogene** → **high-risk category**, irrespective of age, stage, or histology.
 4. **Ploidy of the tumor cells:** It is of **prognostic value in children younger than 2 years** and loses its prognostic significance in older children.
- Amplification of the *N-MYC* oncogene: High-risk neuroblastoma.

Laboratory Finding

Neuroblastoma: Raised urine levels of the metabolites of catecholamines

- Vanillylmandelic acid (VMA)
- Homovanillic acid (HVA).
- **Majority (~90%) of neuroblastomas, secrete catecholamines** (similar to pheochromocytomas) → raised blood levels of catecholamines (hypertension is less frequent).
- **Raised urine levels of the metabolites vanillylmandelic acid (VMA) and homovanillic acid (HVA).**

Course: It is extremely variable.

Catecholamines are increased in:

- Neuroblastoma
- Pheochromocytoma.

Prognostic Factors

1. **Age and stage:** Children **younger than 18 months of age** have **excellent prognosis** regardless of the stage of the neoplasm.
 2. **Morphology:** It is an **independent prognostic factor**. Accordingly, tumors are divided into favorable and unfavorable histologic subtypes.
3. **Amplification of the *N-MYC* oncogene** → **high-risk category**, irrespective of age, stage, or histology.
 4. **Ploidy of the tumor cells:** It is of **prognostic value in children younger than 2 years** and loses its prognostic significance in older children.

Pheochromocytoma

Q. Write short note on pheochromocytoma.

- Pheochromocytomas are **neoplasms composed of chromaffin cells**.
- The **tumor cells synthesize and release catecholamines** and some may produce peptide hormones.
- These tumors are the rare cause of surgically correctable **hypertension**.

Pheochromocytoma: Chromaffin cells that synthesize and release catecholamines.

Rule of 10s

- 10% of pheochromocytomas are **extra-adrenal**.
 - They occur in organs of Zuckerkandl and the carotid body.
 - Extra-adrenal pheochromocytomas are called as **paragangliomas**.
- 10% of sporadic adrenal pheochromocytomas are **bilateral**. In pheochromocytomas associated with familial syndromes up to 50% may be bilateral.
- 10% of adrenal pheochromocytomas **metastasize** and are **malignant**.

- Malignancy is more common in extra-adrenal paragangliomas, and tumors developing due to germline mutations.
- 10% of adrenal pheochromocytomas are **not associated with hypertension**.

Pheochromocytoma: Majority is benign, unilateral and occur in adrenal medulla.

Extra-adrenal pheochromocytomas are called as paragangliomas.

Etiology

- About 25% of individuals with pheochromocytomas and paragangliomas harbor a germline mutation in one of six known genes *RET*, *NFI*, *VHL* and three succinate dehydrogenase complex subunit genes, i.e. SDHB, SDHC, and SDHD.

MORPHOLOGY

Gross

- **Size:** Varies and may range from small, circumscribed lesions to large hemorrhagic masses.
- **Weight:** Average 100 g, but may be range from 1–4,000 g.
- Larger tumors are **well-demarcated** and **may** produce a **lobular pattern**. Part of the adrenal gland can be seen over the surface of the tumor.
- **Cut surface:**
 - Small have **yellow tan**.
 - Large show areas of **hemorrhage, necrosis, and cystic changes** and **typically efface the adrenal gland**.
- **Chromaffin reaction:** When the **fresh tumor tissue** is incubated in **potassium dichromate solution**; the **tumor turns dark brown in color** due to oxidation of stored catecholamines. This is termed positive **chromaffin reaction**.

Microscopy (Fig. 25.16)

Zellballen pattern: Feature of the carotid tumor which is a proto-type of parasympathetic paraganglioma.

- **Zellballen pattern:** Tumor consists of **polygonal to spindle-shaped chromaffin cells or chief cells**, clustered with the sustentacular cells **into small nests or alveoli (Zellballen)** separated by a **rich vascular network** (Fig. 25.16).
- **Cytoplasm:** It has a **fine granular appearance** due to the presence of granules containing **catecholamines**. It is best **demonstrated with silver stains**.
- **Nuclei:** They are **round to oval**, with a stippled “**salt and pepper**” chromatin that is characteristic of neuroendocrine tumors.

Electron microscopy: It shows **membrane-bound, electron-dense secretory granules**.

Immunohistochemistry: Neuroendocrine markers (**chromogranin and synaptophysin**) are **positive** in the chief cells. The peripheral sustentacular cells stain with antibodies against S-100, a calcium-binding protein expressed by a variety of mesenchymal cell types.

Criteria for Malignancy

Definitive diagnosis of malignancy in pheochromocytomas: Metastases.

- None of the histologic feature can reliably predict clinical behavior.
- **Histologic features associated with an aggressive behavior and increased risk of metastasis** include:
 - Numbers of mitoses
 - Confluent tumor necrosis
 - Spindle cell morphology
- Capsular and vascular invasion may be found in benign lesions.

Spread

- **Definitive diagnosis of malignancy in pheochromocytomas is made only when they develop metastases.**
- The tumor may metastasize to regional lymph nodes as well as more distant sites, including liver, lung, and bone.

Clinical Course

Hypertension in 90% of patients

- **Paroxysmal episodes:** It is characterized by abrupt, precipitous elevation in blood pressure, associated with

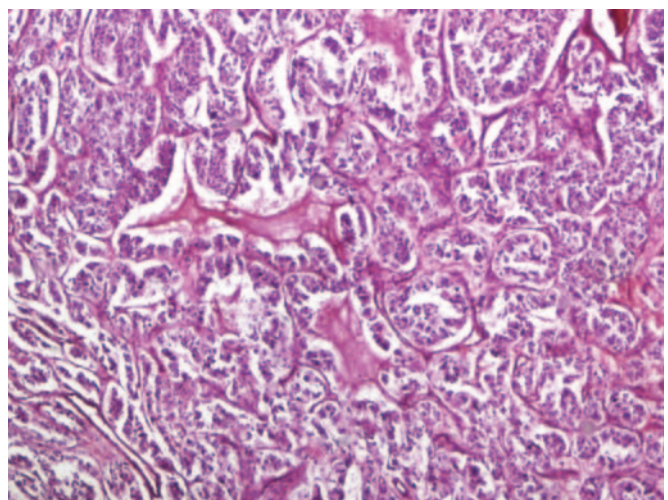


Fig. 25.16: Pheochromocytoma composed of characteristic nests of cells (Zellballen) with abundant cytoplasm

tachycardia, palpitations, headache, sweating, tremor, and a sense of apprehension.

- **Isolated paroxysmal episodes:** Less common.
- **Chronic,** sustained elevation in blood pressure punctuated by paroxysms.

The elevations of blood pressure are induced by the sudden release of catecholamines. This may precipitate congestive heart failure, pulmonary edema, myocardial infarction, ventricular fibrillation, and cerebrovascular accidents.

Complications

Cardiac complications are called **catecholamine cardiomyopathy**, or catecholamine-induced myocardial instability and ventricular arrhythmias.

Laboratory Diagnosis

Demonstration of **increased urinary excretion of free catecholamines and their metabolites**, such as vanillyl-mandelic acid and metanephrines.

Skin Disorders

MELANOCYTIC NEVUS (PIGMENTED NEVUS, MOLE)

Nevus cells: Modified melanocytes.

- The term **mole or nevus** (plural nevi) is used for **any congenital skin lesion** (e.g. a birthmark).
- **Melanocytic nevus is a benign neoplasm (congenital/acquired) of melanocytes** and most nevi are acquired.

MORPHOLOGY (FIG. 26.1)

Q. Write short note on junctional/compound/intradermal nevus.

Nevus types:

1. Junctional
2. Compound
3. Intradermal

- **Progressive changes:** Melanocytic nevi may progress through a series of morphologic changes ranging from junctional nevi to intradermal nevi.
 - **Junctional nevi:** It is the earliest lesions characterized by **aggregates or nests of round nevus cells along the dermoepidermal junction**. These nevus cells have round uniform nuclei with inconspicuous nucleoli, and may show little or no mitotic activity.
 - **Compound nevi:** Most junctional nevi grow into the underlying dermis as nests or cords of cells and form compound nevi.
 - **Intradermal nevi** (Fig. 26.2): **As the compound nevi grow older, the epidermal nests of nevus cells may be lost and retain only the intradermal component to form pure intradermal nevi.**

Clinically, compound and intradermal nevi appear as elevated lesions than junctional nevi.

- **Maturation:** It is a process in which progressive growth of nevus cells occur from the dermoepidermal junction into the underlying dermis.

Maturation of nevus cells is used in differentiating benign nevi from melanomas.

- **Superficial nevus cells** are larger, produce melanin, and grow in nests.
- **Deeper nevus cells** are smaller, produce little or no pigment, and appear as cords and single cells.

This sequence of maturation of nevus cells is of diagnostic use in differentiating benign nevi from melanomas. Melanoma usually does not show maturation.

- Melanocytic nevi are common and may be confused with melanoma.

Clinical Presentation

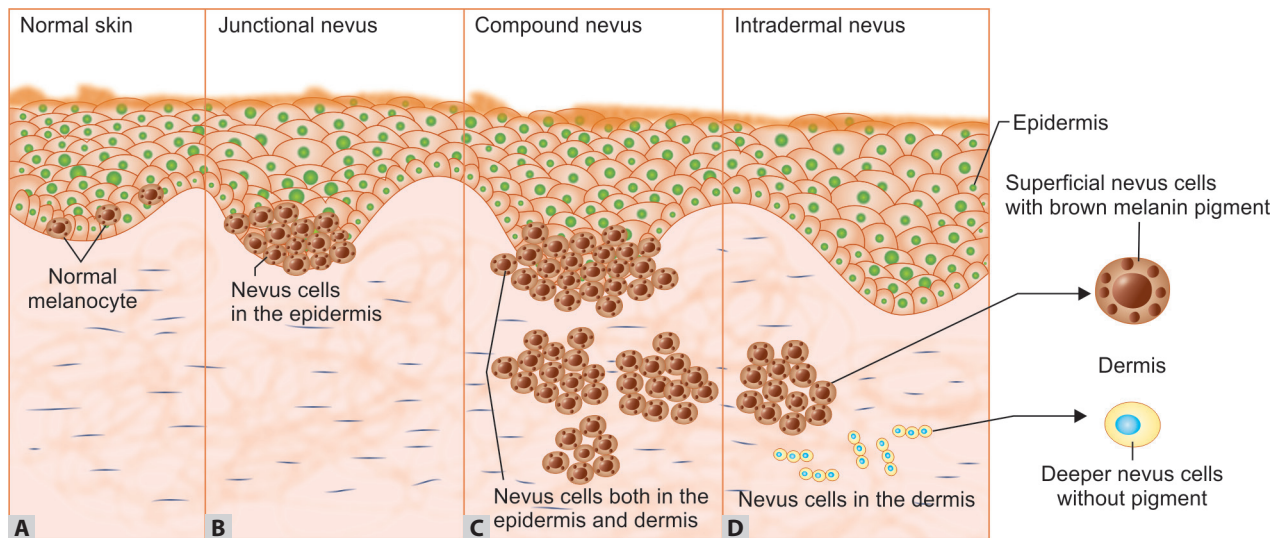
- **Acquired melanocytic nevi** are the **most common** skin lesions.
- **Tan to brown, uniformly pigmented, and small** (usually <6 mm across) lesions.
- **Solid** relatively **flat** (macules) to **elevated** skin (papules) lesions with **well-defined, rounded borders**.

MELANOMA

Q. Write short note on malignant melanoma.

Melanoma is a **relatively common** neoplasm.

- **Site:**
 - **Skin:** It is the most common site and may develop in the trunk, leg, face, sole, palm and nail beds.
 - **Other sites:** Oral and anogenital mucosal surfaces, esophagus, leptomeninges, eye, and the substantia nigra.
- **Cell of origin:** Melanocytes.



Figs 26.1A to D: Maturation sequence of melanocytic nevi: (A) Normal skin with scattered dendritic melanocytes within the epidermal basal cell layer; (B) Junctional nevus; (C) Compound nevus; (D) Intradermal nevus

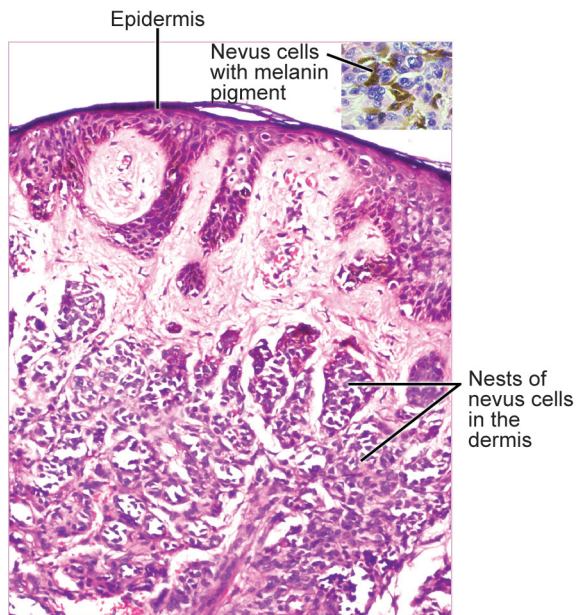


Fig. 26.2: Melanocytic nevus, intradermal type showing nests of nevus cells in the dermis. Inset showing nevus cells with melanin pigment

Etiology and Pathogenesis

Predisposing Factors

Sun Exposure

Melanoma: Excessive exposure to sunlight at early age is the most important risk factor.

- **Melanomas most commonly develop on sun-exposed surfaces**, particularly the upper back in men and the back and legs in women.
- **Lightly pigmented individuals are at greater risk** than darkly pigmented individuals.
- Other environmental factors may also contribute to risk.

Inherited Genes

About 10–15% of melanomas are familial and the genetic abnormalities are as follow:

1. **Mutations in tumor suppressor gene:**
 - **Mutations in *CDKN2A* gene:** It is observed in familial as well as in few sporadic melanomas.
 - *CDKN2A* is a complex locus, which encodes three different **tumor suppressors** namely, 1) *p15/INK4b*, 2) *p16/INK4a* and 3) *p14/ARF*.
 - *CDKN2A* gene is mutated in ~ 40% of familial melanoma and ~10% of sporadic melanomas. These mutations result in increased melanocytic proliferation and escape from oncogene-induced cellular senescence.
 - **Mutations in *RB* gene:** These are common in both familial and sporadic melanomas.
 - **Silencing of *PTEN* gene:** *PTEN* normally downregulate PI-3K/AKT signaling (Fig. 25.11). *PTEN* is **epigenetically silenced** in ~ 20% of melanomas, which leads to **activation of PI-3K/AKT signaling and proliferation of cells**.
2. **Oncogene activation:** It may occur by **mutations in proteins involved in signal transduction**. **RAS** is a

normal signal transduction protein, which stimulates downstream regulator RAS/RAF/MAP kinase cascade resulting in cell proliferation (refer Figs 7.20 and 25.11). Aberrant increases in RAS signaling may occur due to **mutations in RAS or BRAF**, which promote cell growth and survival.

- **Activating mutations in NRAS** (which is one type of RAS) leads to melanoma in 10–15% of tumors.
- **Activating mutations in BRAF** are seen in 60–70% of melanomas. *BRAF* (one of the member of RAF family) is an **intermediate signaling component in the MAP kinase pathway** (refer Fig. 25.11).

3. Mutations that activate telomerase

Malignant melanoma: Mutations in

- *CDKN2A*
- *RB*
- *NRAS*
- *BRAF*.

MORPHOLOGY (FIG. 26.3)

Q. Write short note on morphology of malignant melanoma.

Growth phases

Melanoma: Two growth phases.

- Initial radial growth
- Later vertical growth phase.

1. **Radial growth phase:** During this phase, the **melanoma spread horizontally within the epidermis and superficial dermis**.
 - It represents the **initial stage** where the tumor cells **lack the capacity to metastasize**.
 - Tumors in radial growth phase **fall into different clinico-pathologic classes**.
 - **Lentigo maligna:** It usually **present as an indolent lesion on the face of older men**. The tumor may remain in the radial growth phase for **several decades**.

- **Superficial spreading:** It is the **most common type** of melanoma, and **usually involves the skin exposed to sun**.
- **Acral/mucosal lentiginous melanoma:** It is **not related to sun exposure** and usually seen in the **palm, sole or mucosa**.

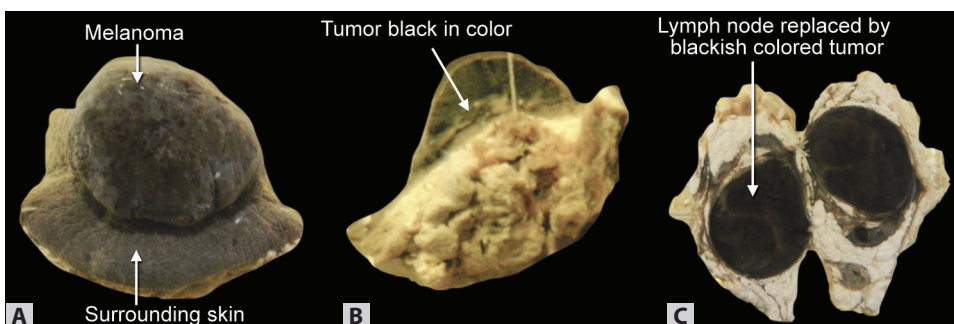
Melanoma radial growth phase: Spreads laterally in the papillary dermis with no metastatic potential.

2. **Vertical growth phase:** After a **variable and unpredictable period**, melanoma from the radial phase develops a **vertical growth phase**.
 - **Characteristics:** During this phase:
 - Tumor cells **invade downward** into the deeper dermis.
 - **Melanoma may appear as a nodule and develop clone of cells with metastatic potential**.
 - **Maturation is absent from the deep invasive portion** of melanoma.
 - **Risk of metastasis** correlates with the **depth of invasion**, which is the distance from the superficial epidermal granular cell layer to the deepest intradermal tumor cells. This measurement is known as the **Breslow thickness**.

Melanoma vertical phase: Final phase penetrates reticular dermis with metastatic potential.

Microscopy (Fig. 26.4)

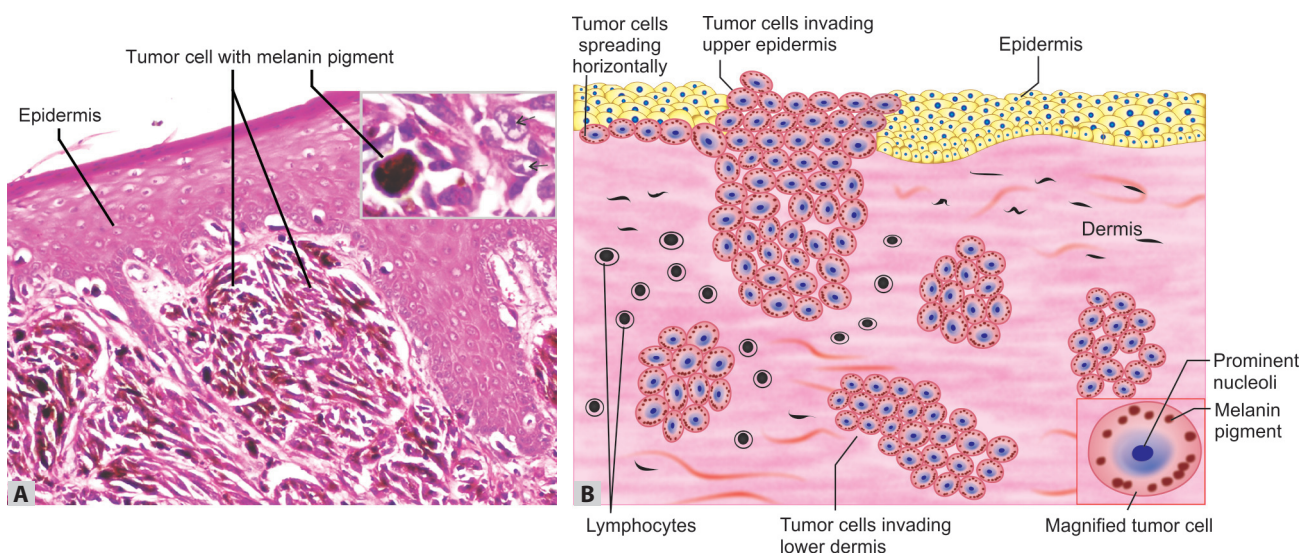
1. **Tumor cells:** They have **similar appearance in both the radial and vertical phases** of growth.
 - **Size:** Tumor cells are usually **larger than normal melanocytes** or nevus cells found in melanocytic nevi.
 - **Nuclei:** They are **large with irregular contours, clumping of chromatin at the periphery** of the nuclear membrane, and **prominent red (eosinophilic) nucleoli**. **Mitotic figures** are often seen.



Breslow thickness:

- Determines the risk of metastasis
- Measurement for depth of invasion
- Distance from the superficial epidermal granular cell layer to the deepest intradermal tumor cells.

Figs 26.3A to C: Gross features of malignant melanoma: (A) Nodular growth projecting from the skin; (B) Cut section of the same shows blackish pigmentation in the tumor; (C) Lymph node with extensive metastasis from malignant melanoma (black color)



Figs 26.4A and B: (A) Photomicrograph of malignant melanoma showing nests of tumor cells in the upper dermis containing melanin pigment. Inset shows tumor cell with melanin pigment; (B) Diagrammatic microscopic appearance of malignant melanoma showing nests of tumor cells infiltrating epidermis (radial growth phase) and dermis (vertical growth phase)

Malignant melanoma:

- Highly aggressive
- Small tumors can produce deadly metastases
- Can metastasize any site.

2. **Pattern of growth:** Tumor cells are arranged in **solid masses, sheets, islands**, etc. Tumor invades upper epidermis as well as deeper dermis.
3. **Melanin pigment:** It is **seen in melanoma**. The melanoma, which **does not show the pigment**, is known as **amelanotic melanoma**. Melanin is present in the **cytoplasm as uniform brown fine granules**.
4. **Tumor infiltrating lymphocytes (TILs):** They may be seen in the **tissue surrounding the tumor**.

Special stain for melanin: **Masson–Fontana or DOPA-oxidase reaction**.

HMB 45 is a tumor marker for: **Malignant melanoma**.

Clinical Features

- Melanoma of the skin is **usually asymptomatic**, but may present with itching or pain at the site of lesion.
- **Changes in pigmented lesions:** These are the **most important clinical signs** of melanoma and include:
 - **Color:** Unlike benign nevi, **melanomas show variations in color**, ranging from shades of black, brown, red, dark blue, and gray.
 - **Size:** Majority are **larger than 10 mm** in diameter at the time of diagnosis. But, if a pigmented lesion is greater

than 6 mm diameter, any change in appearance, and new onset of itching or pain should raise the suspicion of malignancy.

- **Shape:** The borders of melanomas are **irregular and often notched**, whereas they are smooth, round, and uniform in melanocytic nevi.

Q. What is ABCD of melanoma?

ABCD warning signs of melanoma:

- Asymmetry
- Irregular borders,
- Variegated color
- Increased diameter.

Prognostic Factors (Table 26.1)

Prognosis of melanoma: Depth of invasion is most important factor.

TABLE 26.1: Prognostic factors in malignant melanoma

Prognostic factors	Favorable prognosis
• Tumor depth (the Breslow thickness)	• Tumor depth of less than 1.7 mm
• Number of mitoses	• No or very few mitoses
• Evidence of tumor regression (may be due to the host immune response)	• Absence of regression

Contd...

Contd...

• Presence and number of tumor infiltrating lymphocytes (TILs)	• Brisk TIL response
• Gender	• Female gender
• Location (central body or extremity)	• Location on an extremity

Sentinel Lymph Node Biopsy

Sentinel lymph node: It is the initial site of drainage of intratumoral lymphatic vessels.

- Most melanomas metastasize to regional lymph nodes.
- As in breast cancer, sentinel lymph node biopsy may provide additional prognostic information. **Presence of even small number of melanoma cells** (micrometastases) indicates a **worse prognosis**.
- The degree and number of lymph nodes involved correlate well with overall survival.

PREMALIGNANT AND MALIGNANT EPIDERMAL TUMORS

Actinic Keratosis

Actinic keratosis:

- Develop on sun-exposed skin
- Cytological atypia in lowermost epidermis
- Infrequently progress to carcinoma in situ.
- Malignant epidermal tumors may be preceded by precursor lesions, similar to squamous cell carcinoma of the uterine cervix.
- In the skin, these **precursor lesions** are known as **actinic keratoses**; which usually develop in sun-damaged skin.
- Occur more frequently in **light-pigmented individuals**.
- Exposure to **ionizing radiation**, **industrial hydrocarbons** and **arsenicals** may produce similar lesions.

MORPHOLOGY

Gross

- **Size:** It usually less than 1 cm in diameter.
- **Color:** Tan-brown, red, or skin-colored.
- **Consistency:** Rough, sandpaper-like consistency.
- **Sites:** Sun-exposed sites, such as face, arms, dorsum of hands.

Microscopy

- **Cytologic atypia** is seen in the **lowermost basal cells of the epidermis** and these atypical cells have pink or reddish cytoplasm.
- **Superficial dermis** show **thickened, blue-gray elastic fibers** (elastosis).

Squamous Cell Carcinoma

Q. Write short note on squamous cell carcinoma of skin.

- **Second most common tumor** arising on sun-exposed sites.
- **Sex:** More common in men than in women.
- **Clinical presentation:** Appear as sharply defined, red, scaling plaques.

Etiology and Pathogenesis

Squamous cell carcinoma of skin—etiological factors:

- Exposure to UV light
- Chronic immunosuppression
- Chronic non-healing ulcers and scars.

Risk Factors

- **Exposure to UV light:** It may produce **DNA damage** and is the **most important cause** of squamous cell carcinoma of skin. The risk is proportional to the degree of lifetime sun exposure.
- **Chronic immunosuppression:** It may be due to **chemotherapy or organ transplantation** and may contribute to carcinogenesis. The immunosuppression reduces host surveillance and increases the susceptibility of keratinocytes to infection and transformation by oncogenic viruses. These oncogenic viruses include human papilloma virus (HPV) subtypes 5 and 8. Apart from damaging DNA, sunlight can cause a defect in cutaneous immunity by reducing the immune surveillance function of epidermal Langerhans cells.
- **Other risk factors:**
 - **Industrial carcinogens:** Tars and oils.
 - **Chronic non-healing ulcers:** e.g. chronic osteomyelitis.
 - **Old burn scars** (e.g. Marjolin's ulcers).
 - **Ingestion of arsenicals.**
 - **Ionizing radiation.**
 - Tobacco and betel nut chewing in the oral cavity.

Genetics of Squamous Cell Carcinoma

- **Mutations in tumor suppressor *TP53* gene:**
 - **UV light** present in the sunlight damages DNA. Squamous cell carcinoma induced by sunlight, ***TP53* mutation** occurs as an **early event**. **Cells with DNA damage are arrested in the G_1 phase** of the cell cycle by normal ***TP53*** → DNA may be either repaired by DNA repair genes or cells undergo apoptosis if the damage is beyond repair.
 - Mutated ***TP53*** results in **loss of these protective functions** and DNA mutations induced by UV light are passed down to daughter cells.

- **Mutations in DNA repair genes:**
 - **Xeroderma pigmentosum** is a disorder characterized by **inherited mutations in DNA repair genes**, and these patients are susceptible to **squamous cell carcinoma**.
- **Other genes:** Dysregulated RAS signaling may also be responsible.

Xeroderma pigmentosum:

- Inherited mutations in DNA repair genes
- Susceptible to
 - Squamous cell carcinoma
 - Basal cell carcinoma.

MORPHOLOGY

Squamous cell carcinoma in situ

- Characterized by **cells with atypical** (enlarged and hyperchromatic) **nuclei involving all levels of epidermis**.
- **No invasion** through the basement membrane of the dermoepidermal junction.

Invasive squamous cell carcinoma

Gross

Invasive lesions are more advanced lesions, which appear as **nodular growth and may ulcerate**. The ulcer is **surrounded by a wide, elevated, indurated border**.

Microscopy

Tumor consists of **irregular masses of epidermal cells** that **proliferate downward into the dermis**. They show variable degrees of differentiation, ranging from well to poorly differentiated.

Well-differentiated squamous cell carcinoma: Epithelial or squamous pearls.

- **Well-differentiated squamous cell carcinoma** (Fig. 26.5): They are composed of **polygonal squamous tumor cells arranged in orderly lobules** and **produce large amounts of keratin**. Some of this keratin form **epithelial or squamous pearls** and are characteristically seen in well-differentiated tumors.
- **Moderately differentiated squamous cell carcinoma:** They consist of **anaplastic squamous cells, which show single-cell keratinization** (dyskeratosis). It may be associated with areas of geographic necrosis.

Poorly differentiated tumor: May require immunohistochemical stains for keratins to confirm the diagnosis.

- **Poorly differentiated squamous cell carcinoma:** They consist of **highly anaplastic cells**. Poorly differentiated tumor may require immunohistochemical stains for keratins to confirm the diagnosis.

Basal Cell Carcinoma

Q. Write short note on basal cell carcinoma (rodent ulcer) of skin.

- Basal cell carcinoma is the most common, **slow-growing invasive cancer** that **rarely metastasizes**.

- **Sites:** Occur at **sun-exposed sites** and in fair-skinned people. Usual site is **above a line drawn from angle of mouth to the pinna of the ear**.
- **Origin:** Basal cell carcinoma derives its name from the cytological similarity of the tumor cells to the normal basal cells of the epidermis. It is believed to be **primitive 'adnexal' carcinomas probably of indubitable hair follicle derivation**.

Etiology and Pathogenesis

- The incidence of basal cell carcinoma is also **more in patients with immunosuppression** and **inherited defects in DNA repair**, such as **xeroderma pigmentosum**.

Genetics of Basal Cell Carcinoma

Majority of basal cell carcinomas have mutations which causes unbridled hedgehog signaling

Basal cell carcinoma: Mutations in

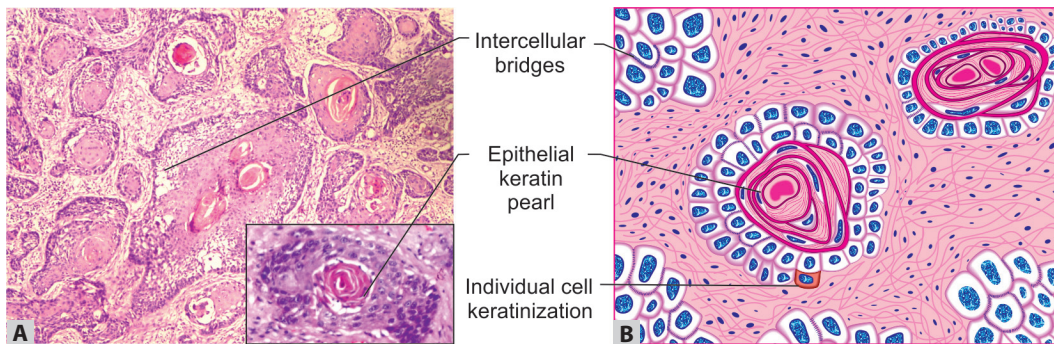
- *PTCH*
- *TP53*
- *DNA repair genes*.

1. **Mutation in tumor suppressor gene:**
 - **Mutation in *PTCH*:** *PTCH* acts as **tumor suppressor gene**. Mutations of genes belonging to the *PTCH* signaling pathway are important for the development of both common **sporadic and inherited** form of basal cell carcinoma.
 - **Sporadic form:** *PTCH* mutations are found in ~30% of sporadic basal cell carcinomas, and may be due to UV damage.
 - **Germline mutation:** **Nevoid basal cell carcinoma syndrome (NBCCS; also known as basal cell nevus or Gorlin syndrome)** is an autosomal dominant disorder characterized by multiple basal cell carcinomas. Patients with NBCCS are born with a germline mutation in one of the *PTCH* alleles; the second normal allele is inactivated in tumors by exposure to mutagens (such as UV light).
 - ***TP53* mutations:** They occur in 40–60% of cases.
2. **Defect in DNA repair genes:** **Xeroderma pigmentosum** is a disorder of DNA repair, associated with increased incidence of basal cell carcinoma.

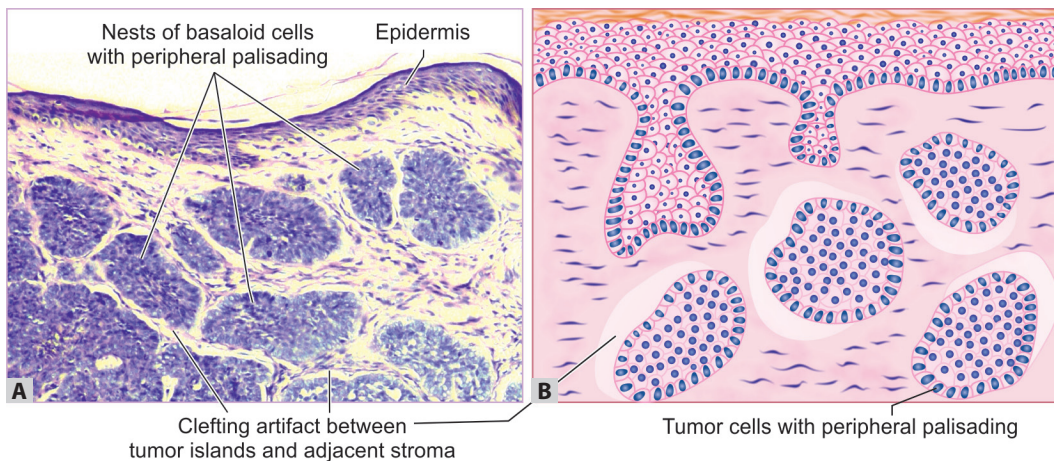
Clinical Presentation

Basal cell carcinoma:

- Erodes the underlying tissue like a rodent
- Locally invasive
- Metastasis very rare.
- Appear as **pearly papules** often containing prominent, dilated subepidermal blood vessels.
- Some tumors may contain melanin and may resemble melanocytic nevi or melanomas.



Figs 26.5A and B: (A) Photomicrograph; (B) Diagrammatic; Well-differentiated squamous cell carcinoma composed of polygonal squamous tumor cells arranged in orderly lobules and produce large amounts of keratin. Some of this keratin form epithelial or squamous pearls (inset of A)



Figs 26.6A and B: Basal cell carcinoma. Composed of nests of uniformly atypical basaloid cells within the dermis, that are often separated from the adjacent stroma by thin clefts: (A) Photomicrograph; (B) Diagrammatic

- Advanced tumors may ulcerate, and locally invade and erode the underlying bone or facial sinuses like a rodent and are known as rodent ulcers.

MORPHOLOGY

Basal cell carcinoma: Usual site is above a line drawn from angle of mouth to the pinna of the ear.

Gross

- Appearance varies and may be nodular, ulcerative, superficial or erythematous.
- Nodulo-ulcerative basal cell carcinoma is the most common type and present as a nodule that increases slowly in size and undergoes central ulceration. A typical lesion consists of a slowly enlarging ulcer surrounded by a pearly, rolled border. This represents the so-called rodent ulcer (erodes the underlying structures similar to a rodent).

Microscopy (Fig. 26.6)

Basal cell carcinoma: Resemble the normal basal cell layer of the epidermis and show peripheral palisading.

- Tumor cells:** They resemble the normal basal cell layer of the epidermis and referred to by some as basaloma (germinative) cells. The tumor cells are deeply basophilic epithelial cells and have a large, oval, or elongated nucleus with narrow rim of cytoplasm.
- Arrangement:** Tumors cells are arranged in nests and are attached to the epidermis and protrude into the subjacent papillary dermis.
- Peripheral palisading:** At the periphery of each nest, the columnar cells are arranged radially with their long axes in parallel alignment known as peripheral palisading.
- Clefting artifact between tumor islands and adjacent stroma:** It may sometimes help distinguish BCC from other adnexal neoplasms displaying basaloid cell proliferation.
- Two patterns of growth:**
 - Multifocal growths:** These tumors originate from the epidermis and extend over several square centimeters or more of skin surface (multifocal superficial type).
 - Nodular lesions:** These tumors grow deeply downward into the dermis as cords and islands of variably basophilic cells with hyperchromatic nuclei.

Bone and Joint Disorders

HEALING OF A FRACTURE

Q. Describe the healing of fractures in long bones.

Phases of Fracture Healing (Fig. 27.1)

Fracture healing: Three phases

1. Inflammatory
2. Reparative
3. Remodeling.

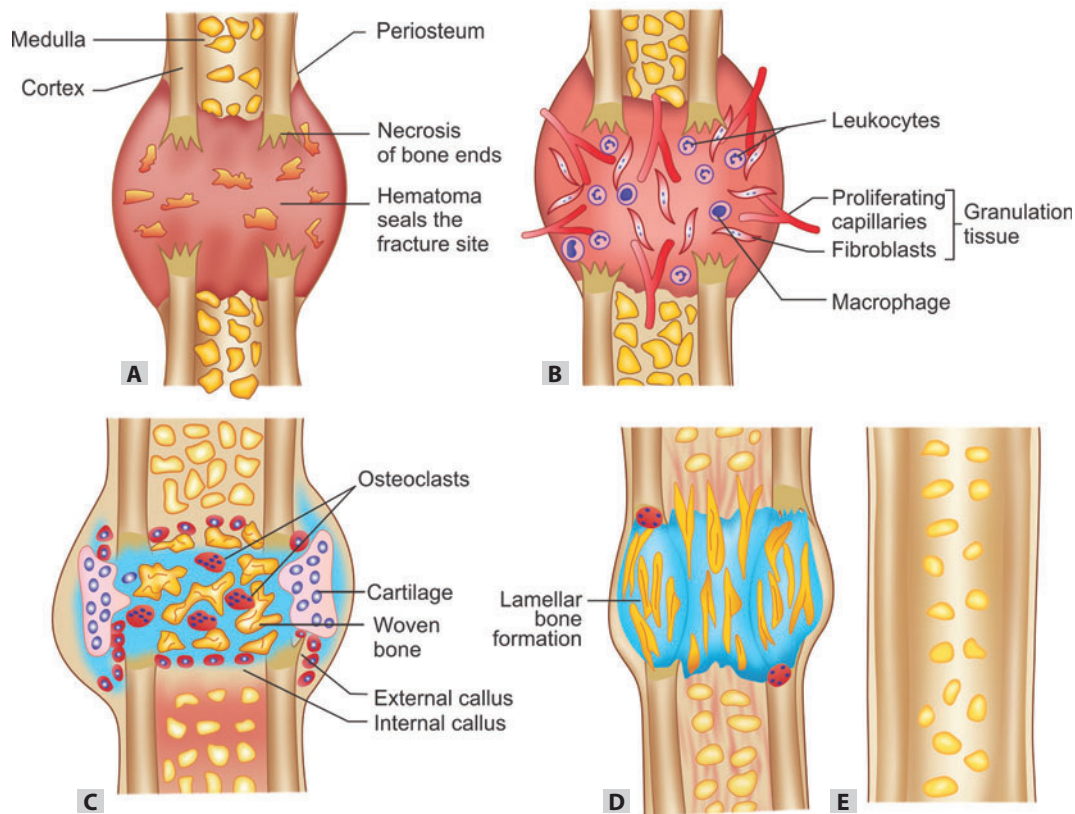
Bone can repair by reactivating processes that normally takes place during embryogenesis. There are three major phases of fracture healing.

Inflammatory Phase

Inflammatory phase

- Fracture and inflammatory cells
- Granulation tissue formation.
- **Fracture and hemorrhage:**
 - Soon **after fracture**, **blood vessels** (in the periosteum, cortex and medullary cavity) **rupture** which leads to extensive hemorrhage (hematoma) at the fracture site and surrounding tissue.
 - **Necrosis of bone** also occurs at the fracture site.
- **Inflammatory cells:**
 - **Fibrin meshwork** in the clotted blood **helps**
 - ♦ To seal the fracture site
 - ♦ Influx of inflammatory cells (neutrophils and macrophages) to the area
 - ♦ Ingrowth of fibroblasts and new capillary vessels (neovascularization) to the site, producing granulation tissue between the fracture fragments.

- **Activation of osteoprogenitor cells:** The **inflammatory cells and platelets release cytokines** (PDGF, TGF- β , FGF and interleukins) \rightarrow **activate the osteoprogenitor cells** in:
 - ♦ Periosteum
 - ♦ Medullary cavity
 - ♦ Surrounding soft tissues.
- **Formation of granulation tissue:** It consists of proliferating capillaries and fibroblasts and are formed at the site of fractures.
- **Soft-tissue callus or procallus formation:**
 - **Osteoprogenitor cells \rightarrow activate both osteoblastic and osteoclastic activities** at the fracture site.
 - **Osteoblasts** derived from activated osteoprogenitor cells **migrate into the granulation tissue and differentiate into osteoid synthesizing units.** They **deposit large quantities of osteoid collagen in a haphazard pattern** producing **woven bone** (unmineralized bone is called osteoid).
 - **Granulation tissue containing** (mineralized or unmineralized) **bone or cartilage** is termed a **callus**.
 - At this stage, **callus** is predominantly **uncalcified** and is called **soft-tissue callus or procallus**, which **provides a type of temporary connection between the ends of the fractured bones**. However, procallus does not have any structural rigidity for any weight bearing. The callus depending on its site and appearance can be divided into external and internal callus.
 - ♦ **External callus:** It is formed from the osteoprogenitor cells of periosteum and surrounding soft tissue and is found on the surface of the bone. It bridges the fracture site outside the bone and continues to grow inwards toward the fracture site. In this region, the osteoprogenitor cells may also differentiate into



Figs 27.1A to E: Healing of a fracture: (A) Immediately after a fracture, blood clot/hematoma forms at the site of fracture; (B) During the inflammatory phase of fracture healing, the inflammatory cells (neutrophils and macrophages) migrate to the area of fracture and neovascularization develops; (C) The reparative phase of fracture healing is characterized by the formation of a callus near the fracture site; (D) In the remodeling phase, the reactive bone (lamellar or woven) develops; (E) Healing is complete and bone attains its original contour

chondroblasts, which form fibrocartilage and hyaline cartilage around the fracture site.

- ◆ **Internal callus:** It is derived from osteoprogenitor cells of medullary cavity and grows outward toward the fracture site. This bridges the fracture in the region of medullary cavity but in contrast to external callus does not contain cartilage.
- The **repair tissue** attains **maximal thickness at the end of the second or third week** and consists of hyaline cartilage and woven bone.

Callus: Granulation tissue containing (mineralized or unmineralized) bone or cartilage.

Fracture healing: First forms woven bone followed by lamellar bone.

Reparative Phase

- Reparative phase
- Callus formation
 - Lamellar bone deposition.

- **Lamellar bone formation:** As the healing advances, the hyaline cartilage and woven bone of the original fracture **callus are replaced by lamellar bone**. This is stronger and consists of **parallel collagen fibers**.
- **Endochondral ossification:** The replacement process is known as **endochondral ossification** with respect to the hyaline cartilage and **bony substitution** with respect to the woven bone.
- **Bony callus:** At this stage, the **callus is mineralized** (calcified) and is known as **bony (osseous) callus**. As the mineralization proceeds, the stiffness and strength of the callus increases. By the second or third week, controlled weight bearing can be tolerated.

Fracture healing: Mineralized callus is called bony/osseous callus.

Remodeling Phase

Rate of newly synthesized osteoid mineralization is best estimated by: Tetracycline labeling.

Remodeling phase: Remodeling to original bone contour.

- Several weeks after a callus has sealed the bone ends, the remodeling phase begins.
- **Resorption of excess portions of bony callus:** During healing, **excess of bony callus is formed around the fracture site**. As the callus is subjected to weight-bearing forces, the portions of bony callus that are not physically stressed by this weight are **slowly resorbed by osteoclasts**. Thus, the osteoclasts act to remodel bone and decrease the size of callus.
- The remodeling phase **substitutes the trabecular bone with compact bone**.
- Remodeling phase continues till the original bone shape (contour), outline and strength of the fractured bone is re-established.

The **whole process of healing** of a bone fracture usually takes **about 6–8 weeks**.

Major Causes of Delayed Fracture Healing (Table 27.1)

TABLE 27.1: Major causes of delayed fracture healing

Local factors	General factors
<ul style="list-style-type: none"> • Excessive movement of fractured bone during healing process • Infection of the fractured site • Severe local soft-tissue injury • Wide separation of fracture ends • Extensive necrosis of the fractured bone • Poor or impaired blood supply (e.g. tibia, head of femur) 	<ul style="list-style-type: none"> • Old age • Poor general health • Drugs, e.g. corticosteroids

Complications of Healing

Q. Mention the complications of healing of fracture bone.

- **Delayed union and nonunion** of fracture.
- **Pseudoarthrosis:** In case of nonunion, too much movement along the fracture gap can cause cystic degeneration in the callus, creating a false joint or pseudoarthrosis.

Complications of fracture healing:

- Delayed union/nonunion
- Pseudoarthrosis
- Large callus with deformity.
- **Large callus with deformity.**

INFECTIONS—OSTEOMYELITIS

Q. Write short note on etiology of osteomyelitis.

Definition: Osteomyelitis is defined as inflammation of the bone and marrow.

Classification

Types of osteomyelitis

- Pyogenic
- Tuberculous
- Others: Chronic nonspecific osteomyelitis.

- **Primary:** Where bone is the primary solitary focus of disease.
- **Secondary:** Develops as a complication of any systemic infection.

Any infection (bacteria, viruses, parasites, fungi) may cause osteomyelitis, but infections by certain pyogenic bacteria and mycobacteria are the most common.

Pyogenic Osteomyelitis

Q. Write short note on morphology of pyogenic osteomyelitis.

Etiology

Pyogenic osteomyelitis: *Staphylococcus aureus* is the most common pathogen.

Pyogenic osteomyelitis: In sickle cell anemia due to *Salmonella*.

It is **usually caused by bacteria**.

- Most common pathogens are *Staphylococcus* species (*aureus* in 80–90% of the cases).
- **Other organisms:**
 - *Escherichia coli*, *Pseudomonas*, *Klebsiella*, *Neisseria gonorrhoeae*, *Haemophilus influenzae* and *Salmonella* species.
 - *Escherichia coli* in patients with genitourinary tract infections or intravenous drug abusers.
 - **Neonatal period:** *Haemophilus influenzae* and group B streptococci.
 - **Patients with sickle cell disease:** *Salmonella* infection.
- **Mixed bacterial infections:** It is due to direct spread or surgery or open fractures.
- In about 50%, no organisms can be isolated.

Portal of Entry of Organism

Pyogenic osteomyelitis:

1. Hematogenous
2. Direct
3. Spread from adjacent site.

Causative organisms may reach the bone through the **bloodstream, directly** or extend **from a contiguous site**.

1. **Hematogenous spread:**

- Source of organisms may be a **focus of infection anywhere in the body** (e.g. skin pustule or infected teeth and gums, intestinal mucosa).
- **Minor injuries** to the mucosa (vigorous chewing of hard foods, brushing of teeth), or minor infections of the skin, **release these organisms** into the blood → causing temporary **bacteremia** → **reach the bone**.
- In **children** (5–15 years) and **drug addicts** (infected needles), it develops in the long bones.

2. **Direct implantation:** Organisms may enter into bone by penetrating wounds, open fractures, or surgical procedures (staphylococci, streptococci, anaerobic organisms).

3. **Spread from adjacent (contiguous) site:** e.g. infections of the feet may spread into the bone in diabetics.

Location of infection: It varies with age.

- **Children** and with hematogenous spread: **Metaphysis of long bones** (knee, ankle, and hip).
- **Neonate:** Metaphysis, epiphysis, or both.
- **Adult:** Epiphysis and subchondral regions.

Pathogenesis and Morphology (Fig. 27.2)

Q. Write short note on pathogenesis and morphology of pyogenic osteomyelitis.

Osteomyelitis:

- Usually due to hematogenous spread
- Metaphysis is the commonest site involved.

The sequence of events and morphological features in osteomyelitis are described together.

- **Transient bacteremia:** Mild injury or trauma can initiate bacteremia by organisms (e.g. *Staphylococcus aureus*).
- **Infection reaches metaphysis of long bone:** Because in the metaphysis, capillaries form loop → which slows the blood flow → provides time for bacteria to penetrate blood vessel walls and establish infective foci within the marrow.
- **Inflammatory reaction:** Once in bone, the bacteria grow and induce an **acute inflammatory reaction with exudates**.
- **Necrosis of bone:** Exudate increases the pressure on the adjacent vessels and further **decreases the blood supply** → produces **bone necrosis**. The necrotic areas coalesce and allow **further bacterial proliferation**.
- **Formation of sequestrum:**

Sequestrum: Fragment of dead/devitalized necrotic piece of bone embedded in the pus.

- Bacterial **infections and pus spreads into cortex** and collect beneath the periosteum → may **lift the periosteum** → **reduces the blood supply** to the affected region → results in **segmental necrosis of the bone** due to both suppuration and ischemia.
- The fragment of **dead necrotic piece of bone**, which is embedded in the pus, is known as a **sequestrum**.
- **Draining sinus:**
 - The **pus penetrates the periosteum** and leads to a **soft-tissue abscess** → may penetrate the skin → form a **draining sinus**. **Hole formed in the bone** during the formation of a draining sinus is known as **cloaca**.
 - The sinus tract may become epithelialized and may remain open, continually draining pus, necrotic bone, and bacteria.
- **Involucrum:** After first week, chronic inflammatory cells become more numerous and the cytokines released stimulates osteoclastic bone resorption and **deposition of reactive bone in the periphery**. Reactive new bone forms a sheath around the necrotic (segment of devitalized infected bone) sequestrum. This **reactive new bone formed is known as involucrum**.

Involucrum: Reactive new bone around the sequestrum.

MORPHOLOGY

The morphologic features **depend on the stage** (acute, subacute, or chronic) and location of the infection.

Gross

- Sequestrum appears as a fragment of bone with ragged brownish-to-black colored margins.

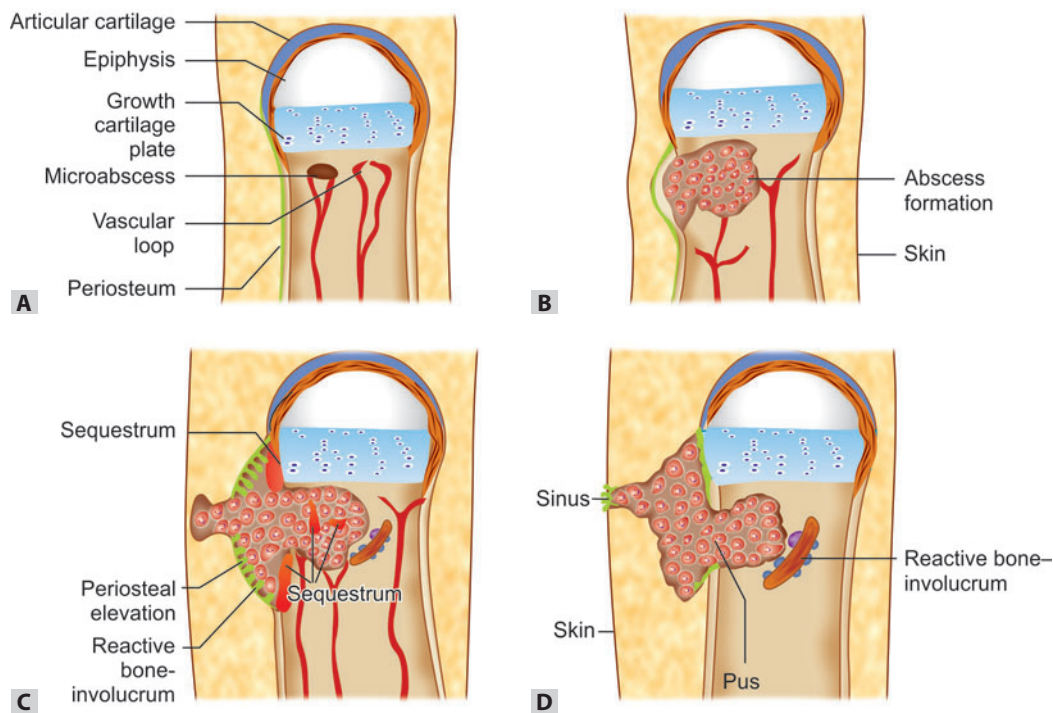
Microscopy

- In the **initial stages**, only the **neutrophils** are seen. As the diseases become chronic, neutrophils admixed with chronic inflammatory cells (lymphocytes and plasma cells) are seen.
- Sequestrum does not show osteocytes and have empty lacunae.

Morphologic Variants of Osteomyelitis

Brodie abscess: Small, solitary, intraosseous abscess localized to the metaphysis surrounded by reactive bone.

- **Brodie abscess:** It is a distinctive form of **subacute pyogenic osteomyelitis**.
 - It appears as a **small, solitary, intraosseous abscess localized to the metaphysis** and is surrounded by reactive bone.
 - It may be **due to inadequate treatment of infection by less virulent organisms**.



Figs 27.2A to D: Pathogenesis of hematogenous osteomyelitis: (A) A small, septic microabscess is formed at the capillary loop; (B) Expansion of the septic focus stimulates resorption of adjacent bony trabeculae. The abscess expands and stimulates reactive bone formation by the periosteum; (C) The abscess, which continues to expand through the cortex into the subperiosteal tissue; (D) The extension of this process into the skin produces a draining sinus. The necrotic bone is called a sequestrum. The viable bone surrounding a sequestrum is termed the involucrum

- **Sclerosing osteomyelitis of Garré:** It is characterized by **extensive new bone formation**, which obscures the underlying structure of the bone and **typically develops in the jaw**.

Complications

Q. Write short note on complications of pyogenic osteomyelitis.

- **Septicemia:** From infection in the bone, organisms may disseminate through the bloodstream and cause septicemia.

Complications of pyogenic osteomyelitis:

1. Septicemia
2. Acute suppurative arthritis
3. Pathologic fractures
4. Squamous cell carcinoma
5. Amyloidosis
6. Chronic osteomyelitis.

- **Acute suppurative arthritis:** Infection may spread through the articular surface into a joint → producing suppurative arthritis → may lead to destruction of the

articular cartilage and permanent disability. It is more common in infants.

- **Pathologic fractures.**
- **Squamous cell carcinoma:** It may arise from the epithelialized sinus tract, rarely sarcoma of bone may develop.
- **Secondary amyloidosis.**
- **Chronic osteomyelitis:** It may develop due to delay in diagnosis, extensive bone necrosis, and inadequate therapy.

Clinical Course

- Present with malaise, **fever, chills, leukocytosis**, and **throbbing pain over the affected region**.
- **Diagnosis**
 - **Radiography:** Lytic focus of bone destruction surrounded by a zone of sclerosis.
 - **Blood cultures** are positive.
 - **Biopsy** and bone cultures.

Tuberculous Osteomyelitis

Q. Write short note on tuberculous osteomyelitis.

Tuberculous osteomyelitis is **usually solitary** but in patients with acquired immunodeficiency syndrome, it is frequently multifocal. It tends to be **more destructive and resistant to control** than pyogenic osteomyelitis.

- **Age:** Usually adolescents or young adults in developing countries.
- **Source of infection:** Pulmonary or extrapulmonary tuberculosis.
- **Predisposing factors:** Diabetes, elderly, immune compromised states and general debility.
- **Route of infection:**
 - **Blood borne:** Usually blood-borne infection, which is from a focus of active pulmonary or extrapulmonary disease.
 - **Direct extension:** From
 - ♦ Lung into a rib.
 - ♦ Tracheobronchial nodes into adjacent vertebrae.
- **Sites:**

Pott disease: Tuberculosis of spine (thoracic and lumbar vertebrae).

- **Spine** (thoracic and lumbar vertebrae) commonly known as **Pott disease**. The infection breaks through intervertebral discs to involve multiple vertebrae and extends down into the soft tissues forming abscesses (**cold abscess—psoas abscess**).
- **Knees and hips.**

MICROSCOPY

It shows dead bone surrounded by **tuberculous granuloma**. The granuloma consists of central area of caseous necrosis surrounded by epithelioid cells and Langhans giant cells, which in turn is surrounded by lymphocytes (Fig. 16.19). The epithelioid cells have a pale-pink granular cytoplasm with indistinct cell boundaries, often appearing to merge into one another. The nucleus is oval or elongate.

Clinical Course

- Low-grade fever with evening rise of temperature.
- Pain on motion, localized tenderness.
- Weight loss.

Complications

Psoas abscess: May be the first manifestation of tuberculous spondylitis.

- **Spine:**
 - **Destruction of vertebrae:** Causes severe **scoliosis or kyphosis** and neurologic deficits due to spinal cord and nerve compression.
 - **Psoas abscess:** Infection from spine may rupture into the soft tissue anteriorly and pus and necrotic debris may drain along the spinal ligaments and form a **cold abscess, i.e. an abscess lacking acute inflammation**. Psoas abscess is the condition in which infection from lower lumbar vertebrae dissects along the pelvis, and appears as a draining sinus of the skin in the inguinal region. It may be the first manifestation of tuberculous spondylitis.
- **Tuberculous arthritis.**
- **Sinus tract formation.**
- **Amyloidosis.**

BONE TUMORS

Q. Classify bone tumors.

Classification of Bone Tumors (Table 27.2)

Bone tumors: Classified according to their normal tissue counterpart.

Osteochondroma

Osteochondroma: Most common benign cartilage-capped bone tumor.

- Osteochondroma (exostosis) is a **most common benign cartilage-capped tumor**, which attaches to the underlying bone by a stalk.
- **Age group:** Late adolescence and early adulthood.
- **Sex:** Men are affected three times more often than women.

MORPHOLOGY (FIG. 27.3)

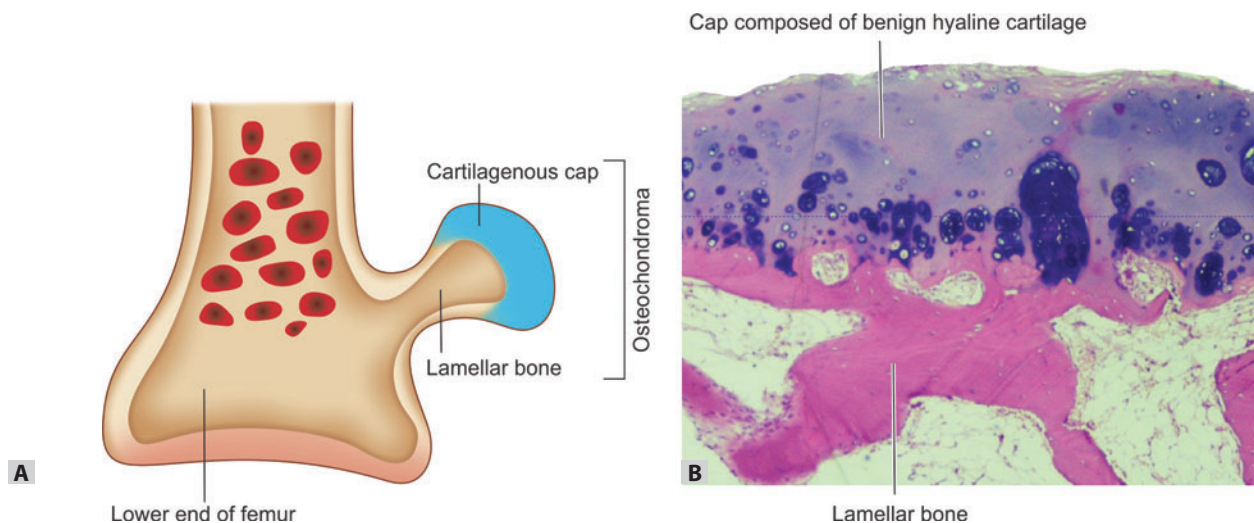
- **Site and location:** Arise from the **metaphysis near the growth plate of long tubular bones**, especially about the knee.
 - Osteochondromas are sessile or mushroom shaped, and range in size from 1 to 20 cm. The cap is composed of benign hyaline cartilage and underlying lamellar bone.

Clinical Features

- Slow-growing masses, detected as an incidental finding.

TABLE 27.2: Major primary tumors of bone

Histologic type	Benign	Malignant
Osteogenic/Bone forming	Osteoma Osteoid osteoma Osteoblastoma	Osteosarcoma (primary/secondary)
Chondrogenic/Cartilaginous	Osteochondroma Chondroma Chondroblastoma	Chondrosarcoma Dedifferentiated chondrosarcoma
Hematopoietic		Myeloma Malignant lymphoma
Unknown origin	Giant-cell tumor (primitive osteoblast precursors) Unicameral cyst Aneurysmal bone cyst	Ewing sarcoma
Neuroectodermal		Chordoma



Figs 27.3A and B: (A) Osteochondroma appears as a mushroom-shaped tumor covered by cartilage; (B) Microscopy of osteochondroma showing cap composed of benign hyaline cartilage and underlying lamellar bone

Osteosarcoma

Q. Write short note on osteosarcoma.

- Osteosarcoma (osteogenic sarcoma—OS) is the **most common (20%) primary malignant bone tumor**, excluding of myeloma and lymphoma.

Definition: Osteosarcoma is a **highly malignant bone tumor** characterized by formation of **bone matrix or osteoid** (unmineralized bone) by **malignant tumor cells**.

Osteosarcoma: Most common primary malignant bone tumor.

- Age group:** OS has a **bimodal age distribution**.
 - Three-fourth occur in between **10 and 20 years age**.
 - Secondary OS** (following **Paget disease, bone infarcts, irradiation, etc.**) develop in **elderly after 40 years of age**.
- Sex:** Affects boys more commonly than girls (2:1).

Pathogenesis

Based on the pathogenesis, OS is divided into primary and secondary.

Primary osteosarcomas

In this type, the underlying bone is unremarkable. About 70% of OS have acquired genetic abnormalities, such as **ploidy changes and chromosomal aberrations**.

- Mutations in tumor suppressor gene:**
 - RB, the retinoblastoma gene** (a cell cycle regulator) mutations is associated with 1000-fold increased risk of OS and its mutation is found in about two-thirds of patients.
 - Patients with Li-Fraumeni syndrome** (germline **TP53 mutations**) have a greater incidence of OS.

- **Abnormalities in *INK4a***, which codes p16 (a cell cycle regulator) and p14 have also been found in OS.
- **MDM2 and CDK4:** They are cell cycle regulators that inhibit functions of p53 and RB, respectively. They are overexpressed in many low-grade OS.

Osteitis deformans (Paget disease of bone):

- Mosaic pattern of lamellar bone histology
- Increased risk of OS.

Secondary Osteosarcoma

Osteosarcomas in older persons almost always develop in association with pre-existing bone disorders. These include:

- **Paget disease of bone.**
- **Radiation exposure:**
 - Following therapeutic radiation for tumor, such as lymphoma.
 - Radium watch dial painters, who wetted their brushes by licking them, developed OS many years later.
- **Chemotherapy:** Children treated with **alkylating agents** (other malignancies) have an increased risk.
- **Pre-existing benign bone lesions:** e.g. **fibrous dysplasia**, **osteomyelitis**, and bone marrow infarcts. Trauma may call attention to an existing OS rather than causing the tumor.

Classification

Q. Write short note on morphology and radiological appearance of OS.

It can be classified in different ways:

1. **Anatomic site of origin**
 - **Conventional** (classical, medullary, intramedullary, textbook)
 - **Intracortical**
 - **Surface OS**
 - Parosteal (juxtacortical): They arise on the surface of the cortex.
 - Periosteal: They arise on the surface of periosteum.
2. **Degree of differentiation:**
 - Well differentiated
 - Moderately differentiated
 - Poorly differentiated.
3. **Number of tumors**
 - **Solitary**
 - **Multicentric**
 - Synchronous
 - Metachronous.

4. **Pathogenesis** (described above)
 - **Primary**
 - **Secondary** to pre-existing disorders.
5. **Histologic features** (described below).

MORPHOLOGY

Most Common Subtype

- Primary
- Solitary
- Conventional/Intramedullary
- Poorly differentiated.
- **Location:** Usually arise in the **metaphyseal region of the long bones** of the extremities.
- **Sites:** It usually arises near the knee or shoulder. The common site is:
 - Lower femur
 - Upper tibia, or fibula
 - Proximal humerus.

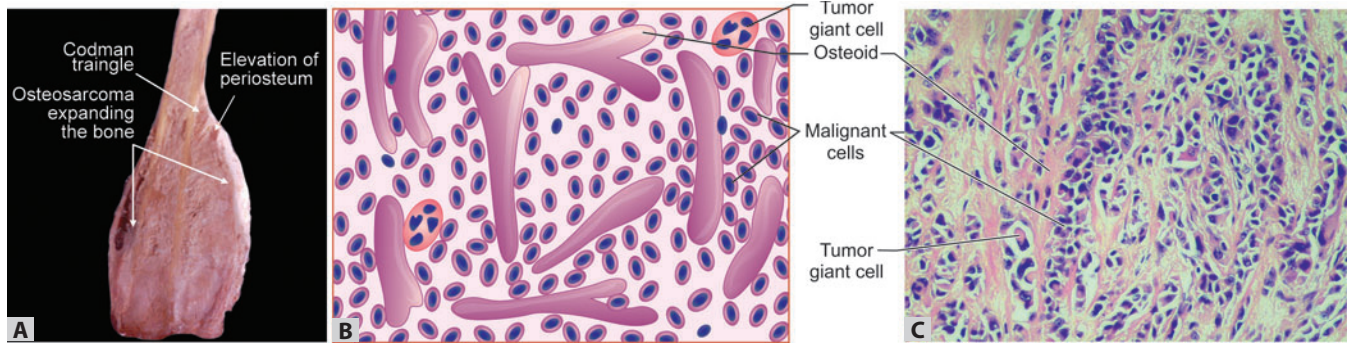
Gross (Fig. 27.4)

- Gross appearance **varies depending on the proportions of bone, cartilage, stroma and blood vessels.**
- **Size:** Usually **big bulky tumors.**
- **Cut surface:** **Gray-white** in color, **gritty**, shows **areas of hemorrhage and cystic degeneration.**

Microscopy (Fig. 27.4)

Osteosarcoma: Single diagnostic feature is production of osteoid or bone by malignant tumor cells.

- **Malignant tumor cells:**
 - Tumor cells vary in size and shape
 - Nucleus: Usually show **large hyperchromatic nuclei** and often shows **mitotic figures**
 - Bizarre tumor giant cells are common.
- **Matrix component:** Production of **osteoid** (unmineralized/noncalcified) or **bone** (calcified osteoid) by malignant tumor cells is the single **diagnostic feature** of conventional OS.
 - ♦ **Osteoid:** It appears as a **dense, uniform, eosinophilic glassy intercellular material.**
 - ♦ **Neoplastic bone:** It usually has a coarse, lace-like architecture.
- **Histological subtype:** Most tumors contain a **mixture of cells with varying amounts of matrices** mentioned below. Depending on the one predominant matrix, conventional OS is divided into:
 - **Osteoblastic:** Large amount of osteoid and bony trabeculae.
 - **Chondroblastic:** Abundant malignant cartilage.
 - **Fibroblastic/fibrohistiocytic:** They appear similar to malignant fibrous histiocytoma.
 - **Telangiectatic:** Large cavernous dilated vascular channels and it is more aggressive.



Figs 27.4A to C: (A) Gross appearance of osteosarcoma of the lower part of distal femur showing a tan-white tumor, filling most of the medullary cavity of the metaphysis and proximal diaphysis. The tumor has infiltrated through the cortex, lifted the periosteum and this region on X-ray appears as Codman triangle; (B) Diagrammatic; (C) Photomicrograph of microscopy of osteosarcoma composed of pleomorphic tumor cells separated by osteoid

- **Small cell:** The tumor cells are small in size, uniform and simulate the appearance of Ewing's sarcoma and malignant lymphoma.
- **Giant cell:** This subtype contains numerous giant cells.
- Vascular invasion and necrotic area may be conspicuous.
- **Immunohistochemistry:** The malignant tumor cells stain prominently for alkaline phosphatase and osteonectin.

Osteosarcoma: Histological subtypes

1. Osteoblastic
2. Chondroblastic
3. Fibroblastic/Fibrohistiocytic
4. Telangiectatic
5. Small cell
6. Giant cell.

Osteosarcoma: Stain positive for

- Alkaline phosphatase
- Osteonectin.

Clinical Course

- Usually present as **painful, progressively enlarging masses** around the knee or other involved site.
- The involved area is **swollen and tender** and the function of adjacent joint becomes reduced.
- **Serum alkaline phosphatase is increased.**

Radiological Appearance

- OS shows a **large destructive, mixed lytic** (bone destruction) and **blastic mass** (neoplastic bone formation) with infiltrative margins.
- **Codman triangle:**

Q. Write short note on Codman triangle.

Osteosarcoma: Codman triangle and sunburst appearance on X-ray.

Codman triangle: Characteristic but not diagnostic of osteosarcoma.

Surface Osteosarcoma

There are two variants:

- **Juxtacortical (parosteal) OS:**
 - A variant of low-grade OS.
 - Usually arises on the surface of the bone and grows along the external surface of the bone, both linearly and longitudinally, without involving the medullary cavity.
 - **Prognosis: Excellent.**
- **Periosteal OS:**
 - A rare variant of OS.
 - Occurs on the periosteal surface of the bone, between the cortex and periosteum.
 - Microscopically, it is high-grade osteosarcoma, and characteristically has predominant cartilage component.

- The tumor frequently infiltrates the cortex and lifts the periosteum, and produces a space between the cortex and lifted ends of periosteum. Radiologically, this space appears as **triangular shadow** and is known as **Codman triangle** (characteristic but not diagnostic of this tumor).
- The space mainly contains reactive new bone, which is arranged perpendicular to the bone surface, but it may also contain malignant tumor.
- **Sunburst appearance:** When the malignant tumor extends into soft tissue, parallel lines of mineral deposition in the periosteal region gives an appearance of rays of sun → called as “sunburst” appearance (Fig. 27.5).

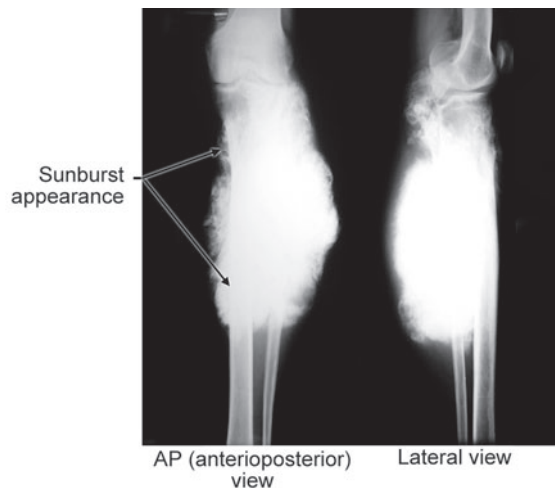


Fig. 27.5: X-ray of the knee joint. Osteosarcoma of proximal tibia extending into the soft tissue with parallel lines of mineral deposition giving rise to sunburst appearance

Spread

- **Local spread:**
 - **Invasion of the adjacent cortex** and destroys the nearby cortex.
 - **Elevation or perforation of the periosteum** by tumor tissue.
 - **Spread along the medullary (marrow) cavity.**
 - **Extension into the epiphysis:**
 - ♦ Articular end of the bone is generally not involved in the initial stage.
 - ♦ Later, it may infiltrate the epiphyseal plate and may even involve the joint space.
 - **Extension into the soft tissues** and may involve skin
- **Blood spread:** It is an aggressive tumor, which may spread through the bloodstream to the **lungs**. Less commonly, it may metastasize to other bones, the pleura, brain, and the heart.

Giant-cell Tumor

Q. Write short note on giant-cell tumor/osteoclastoma.

Definition: Giant-cell tumor (GCT synonym osteoclastoma) is characterized by **multinucleated osteoclast-type giant cells randomly and uniformly distributed in a background of mononuclear cells.**

Giant-cell tumor:

- Neoplastic mononuclear cells
- Reactive multinucleated osteoclast type of giant cells.
- Giant-cell tumor is a **relatively uncommon, locally aggressive and potentially malignant neoplasm.**

- **Age:** Usually seen between **20 and 40 years**. In elderly, it may be secondary to irradiation.
- **Sex:** Slight predilection for women.
- **Cell of origin:** Primitive stromal cells.
- **Pathogenesis:** The neoplastic cells of giant cell tumor are primitive osteoblast precursors and form only a minority of the tumor cells. The major cells of the tumor are non-neoplastic osteoclasts and their precursors. The neoplastic cells express high levels of RANKL. They promote the proliferation of osteoclast precursors and their differentiation into mature osteoclasts through RANK expressed by these cells.

MORPHOLOGY

Site

Giant-cell tumors involve **epiphyses but may extend into the metaphysis of a long bone.**

Bones Involved

Any bone can be involved, majority arise in:

- Knee area: Distal femur and proximal tibia.
- Lower end of radius, humerus and fibula.

Gross (Fig. 27.6 A)

Large tumor, clearly circumscribed and frequently undergo cystic change.

- Cut surface
 - **Soft and red-brown** without bone or calcification
 - **Numerous hemorrhagic areas** give an appearance of a sponge full of blood.

Microscopy (Figs 27.6B and C)

Two types of cells

1. **Mononuclear (stromal) cells:** They are of two types.

- **Neoplastic mononuclear cells:** They are **uniform oval and plump** with **large nuclei, prominent nucleoli** and **scanty cytoplasm**. Tumor mostly consists of these neoplastic cells. Mitotic activity is common in these cells but not found in the giant cells. **Diagnosis of malignancy depends upon the morphology of the mononuclear cells rather than that of the multinucleated giant cells.** The nuclei of these cells and the osteoclasts are similar. Thus, it is difficult to identify the neoplastic osteoblast precursors on routine histology. The tumor cells do not synthesize bone or cartilage.

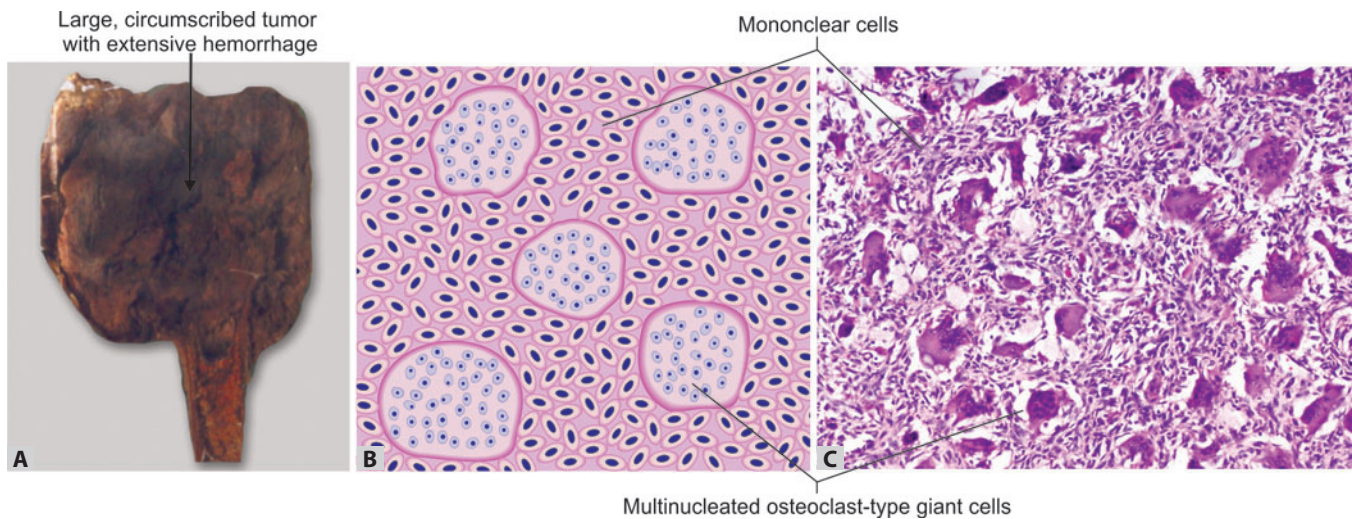
- **Non-neoplastic:** These are mononuclear cells derived from macrophage–monocyte.

2. **Multinucleated (osteoclastic) giant cells:**

- The **background** shows **numerous large osteoclast-type giant cells** throughout the richly vascularized stroma.
- These **cells have 100 or more nuclei**, which resemble those of the mononuclear cells.

Area of cystic degeneration, necrosis and mitotic figures may be seen.

Secondary features: These include areas of **hemorrhage, necrosis, hemosiderin deposition**, and reactive bone formation.



Figs 27.6A to C: Giant-cell tumor of bone: (A) Grossly shows a circumscribed tumor with hemorrhage in the upper end of tibia. Microscopically; (B) Diagrammatic; (C) Photomicrograph, shows numerous multinucleated giant cells. The background shows mononuclear stromal cells

Giant cell lesions of bone and synovium:

- Giant cell tumor
- Brown tumor (hyperparathyroidism)
- Giant cell reparative granuloma
- Chondroblastoma
- Tenosynovial giant cell tumor (previously known as pigmented villonodular synovitis).

Radiological Appearance

Giant-cell tumor: Soap-bubble appearance on X-ray examination.

- It produces a **lytic lesion** and **grows slowly, expands the bone** and **destroys the overlying cortex**.
- The tumor produces a **bulging soft tissue mass surrounded by a thin, shell of reactive bone** due to periosteal reaction. This on radiology has multiloculated or **soap-bubble** appearance (Fig. 27.7).

Clinical Course

GCTs must be considered as potentially malignant.

- Commonly seen near knee joints.
- Pain (usually in the joint adjacent to the tumor) and arthritis-like symptoms.
- Microfractures and pathologic fractures may develop due to thinning of the cortex.

Biologic Behavior

- Majority of GCTs behaves in a **benign fashion**, but **locally aggressive tumors may locally recur**.

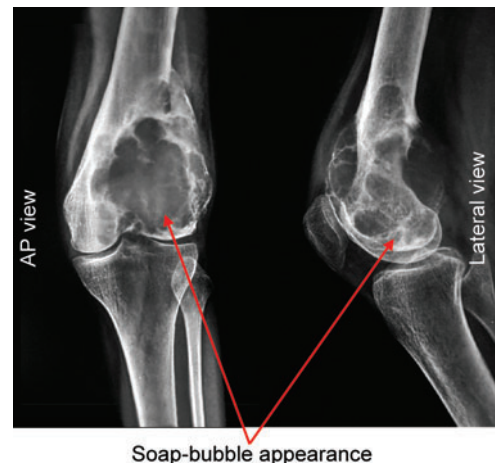


Fig. 27.7: X-ray of the knee joint. Giant cell tumor of distal femur showing soap-bubble appearance

- However, **all GCTs must be considered as potentially malignant**, because they may metastasize.

Spread

- **Local spread:** Tumor is usually restricted within the involved bone surrounded by periosteum.
 - Aggressive tumors may penetrate the cortex and the periosteum, and may involve the joint capsule and the synovial membrane.
- **Hematogenous spread:** Common site is lung.

Chondrosarcoma

Q. Write short note on Chondrosarcoma.

Chondrosarcoma: Malignant mesenchymal tumor forming cartilage.

Chondrosarcomas are **malignant bone tumors** characterized by the **production of neoplastic cartilage**.

- **Age group:** Usually around **40 years or older** (fourth to sixth decades).
- **Sex:** **Males are twice frequently** affected than females (2:1).

Classification

- **According to anatomic site:**
 - **Central (intramedullary) tumors** (about 90%)
 - **Peripheral (juxtacortical and surface):** Less common and arise outside the bone.
- **Microscopic appearance:**
 - Conventional (hyaline and/or myxoid)
 - Clear cell
 - Dedifferentiated
 - Mesenchymal.
- **Presence or absence of pre-existing lesion**
 - **Primary chondrosarcoma:** No known pre-existing lesion.
 - **Secondary chondrosarcoma:** Developing in a pre-existing lesion
 - ♦ Enchondroma
 - ♦ Solitary osteochondroma
 - ♦ Hereditary multiple osteochondromas.

MORPHOLOGY

Central conventional chondrosarcoma: It consists of malignant hyaline and myxoid cartilage.

Location

Chondrosarcomas commonly arise in the **central medullary cavity** of

- Pelvic bones
- Shoulder
- Ribs
- Long bones.

Gross (Fig. 27.8A)

- **Large bulky tumors**, and consists of **nodules of gray-white/light-blue with translucent glistening areas** (represents cartilage).
- **Cut section:**
 - **Mucoid/myxoid feel** and the matrix may ooze from the cut surface.
 - **Spotty calcifications** are common and appear as white gritty areas.

- **Central area of necrosis** may produce cystic spaces.
- Adjacent cortex may show thickening or erosion.

Microscopy

It consists of **malignant cartilage cells** in **various stages of maturity**. It is graded from 1 to 3, depending on the degree of cellularity, cytological atypia and mitotic activity.

- **Low-grade or grade 1** (Fig. 27.8B): Mild hypercellularity.
 - **Cells:**
 - ♦ Chondrocytes show **plump vesicular nuclei** with small nucleoli.
 - ♦ **Binucleate cells** are few in number.
 - ♦ **Mitotic figures** are rare.
 - ♦ **Matrix:** Portions of the matrix frequently mineralize, and the cartilage may undergo endochondral ossification.
- **Grade 2: Moderate cellularity** and more cellular than grade 1. Chondrocytes show more **pronounced nuclear changes**.
- **Grade 3:** They are uncommon and show **marked hypercellularity**.
 - Chondrocytes show **extreme pleomorphism**
 - **Bizarre tumor giant cells**
 - **Mitoses frequent**.

Variants

- **Dedifferentiated chondrosarcoma** is a low-grade chondrosarcoma with a second, high-grade component which do not have cartilage.
- **Clear cell chondrosarcoma** composed of large, malignant chondrocytes with abundant clear cytoplasm, numerous osteoclast-type giant cells, and reactive bone formation within the tumor.
- **Mesenchymal chondrosarcoma** consists of islands of well-differentiated hyaline cartilage surrounded by sheets of small round cells.

Radiological Appearance

Chondrosarcoma: Popcorn calcification, flocculent densities with ring-like ossifications.

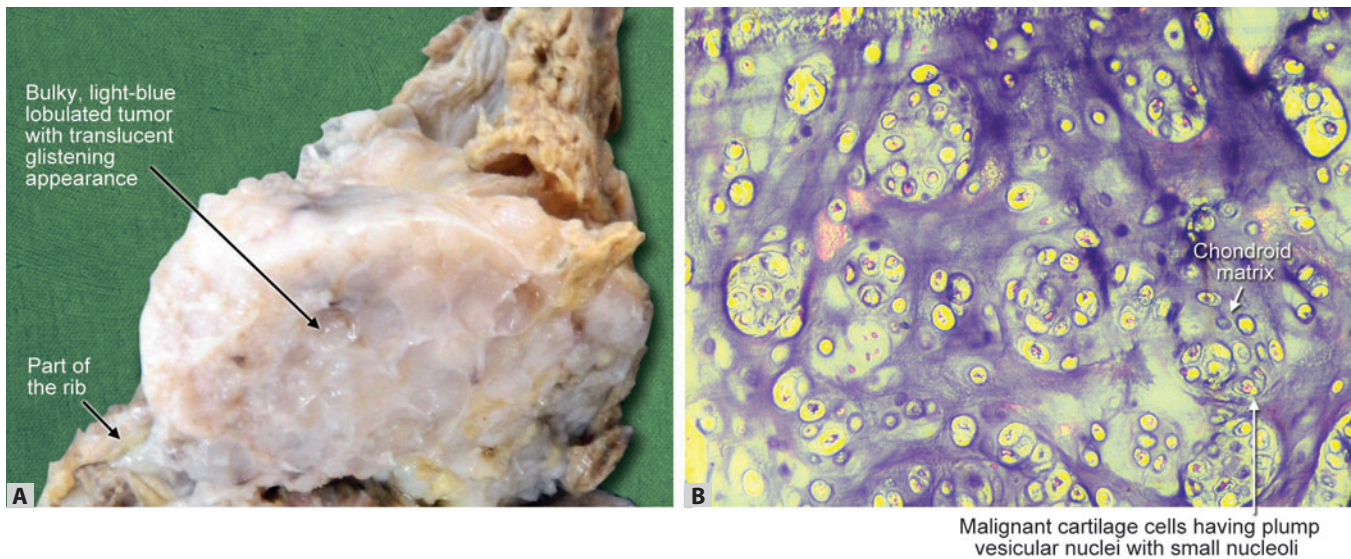
- Shows a tumor in the **medullary cavity** with **poorly defined borders**. The shaft is thickened and tumor perforates the cortex. The calcified matrix appears as foci of **flocculent densities** with **ring-like ossifications**.

Clinical Features

- **Painful, progressively enlarging masses.** Biologic behavior of the tumor depends on the histological grade.

Spread

- **Local spread:** Locally aggressive tumor. It may **infiltrate the marrow space** and may grow with broad pushing fronts into the **surrounding soft tissue**.
- **Hematogenous spread:** Lungs and skeleton.



Figs 27.8A and B: (A) Chondrosarcoma of rib; (B) Microscopic appearance of well-differentiated chondrosarcoma

Ewing Sarcoma/Primitive Neuroectodermal Tumor

Q. Write short note on Ewing sarcoma.

Definition: Ewing sarcoma family of tumors is **primary malignant small round-cell tumors of bone and soft tissue.**

Ewing sarcoma: Highly aggressive small round cell tumor affecting the diaphysis.

- **Uncommon** tumors and constitute ~ 6–10% of primary malignant bone tumors.
- **Age:** Mostly seen between **10 and 15 years** of age, and about 80% are seen in patients younger than 20 years. In children, it is second most common group of bone sarcomas, first being osteosarcoma.
- **Sex:** More frequent in **boys** than girls (2:1).

Categories

- It consists of **two tumors that differ only in their degree of neural differentiation.** This distinction has no clinical significance.
 1. **Ewing sarcoma (EWS): Undifferentiated tumors.**
 2. **Primitive neuroectodermal tumor (PNET):** With neural differentiation.

Etiology and Pathogenesis

- **Origin:** It arises from **multipotent mesenchymal stem cell.**
- **Chromosomal abnormalities.**
 - Translocation of *EWS* gene on chromosome 22 (22q12) with a gene encoding an ETS family transcription factor (*FLI-1* or *ERG* gene).

- The **fusion of the *EWS1* gene to *FLI-1* gene** (more common) resulting in fusion protein, EWS/FLI-1 → produces chimeric/aberrant transcription factors → **activation of C-myc promoter** → abnormal cell proliferation and survival.
- A less common translocation produces *EWS/ERG* fusion gene → produces a variant of EWS with a significantly worse prognosis.

Location of genes:

- *EWS* on chromosome 22q12
- *FL1* gene on 11q24
- *ERG* gene on 21q22.

Ewing sarcoma: Chromosomal abnormalities of *EWS* gene rearrangements

- Translocation of *EWS* gene
- Fusion gene EWS/FLI-1.

Ewing sarcoma: Translocations of diagnostic importance

- t(11; 22) (q24;q12)
- t(21;22) (q22;q12).

MORPHOLOGY

Q. Write short note on morphology of Ewing sarcoma.

Location

Arise in the **diaphysis of the medullary cavity of long bones.**

Site

Ewing sarcoma and PNET involve the **long tubular bones in childhood.**

- **Long bones:** Humerus, tibia and femur.
- **Flat bones:** Pelvis.

Gross

- Arise in the **diaphysis** and **may infiltrate the medullary spaces** without destroying the bony trabeculae.
- **Diffusely infiltrate the cortex** and the tumor **may also penetrate the periosteum** and spread into the soft tissues.
- **Soft, grayish white/tan-white**, and frequently show areas of hemorrhage and necrosis.

Microscopy (Fig. 27.9)

- **Tumor cells:** They are **uniform, small and round**.
 - **Size:** Tumor cells are about **twice the size of a lymphocyte**.
 - **Cytoplasm:** **Scant or little** and **appears clear** because it is **rich in glycogen**, which is an important diagnostic feature. This **stains positive** with the **periodic acid-schiff (PAS) stain**.
- **Arrangement:** **Closely packed sheets** of cells.
- **Homer–Wright rosettes:** They may be seen. They consist of **tumor cells arranged in a circle about a central fibrillary space**. This is **indicative of neural differentiation**.
- **Mitoses** are **infrequent** even though the tumor is highly cellular.

Ewing sarcoma:

1. Uniform small round cells
2. PAS +ve granules in the cytoplasm
3. Homer–Wright rosettes.

- **Interstitial stroma:** **Little or absent** and the fibrous strands separate the sheets of tumor cells into irregular **nests or sheets**. **Necrosis** may be prominent.

Immunohistochemistry

EWS cells express characteristic antigens, some of which are part of the translocation product (e.g. **FLI-1 and CD99**).

Immunohistochemistry in EWS:

- FLI-1
- CD99.

Radiographic Appearance

Ewing sarcoma: Onion-skin appearance on X-ray.

- It produces **destructive lytic lesion** in which the border between normal bone and the tumor is indistinct. The tumor may extend into the surrounding soft tissues.
- Characteristic circumferential, **discontinuous layers of periosteal new bone formation** produces **onion-skin pattern** (Fig. 27.10).

Clinical Features

- **Painful enlarging masses** and the affected site is **tender, warm, and swollen**.
- **Systemic features:** It may develop in some and **mistaken for infection like osteomyelitis**.
 - Fever, weakness, and bone pain.
 - Hematological findings: Anemia, leukocytosis, and raised erythrocyte sedimentation rate.

Spread

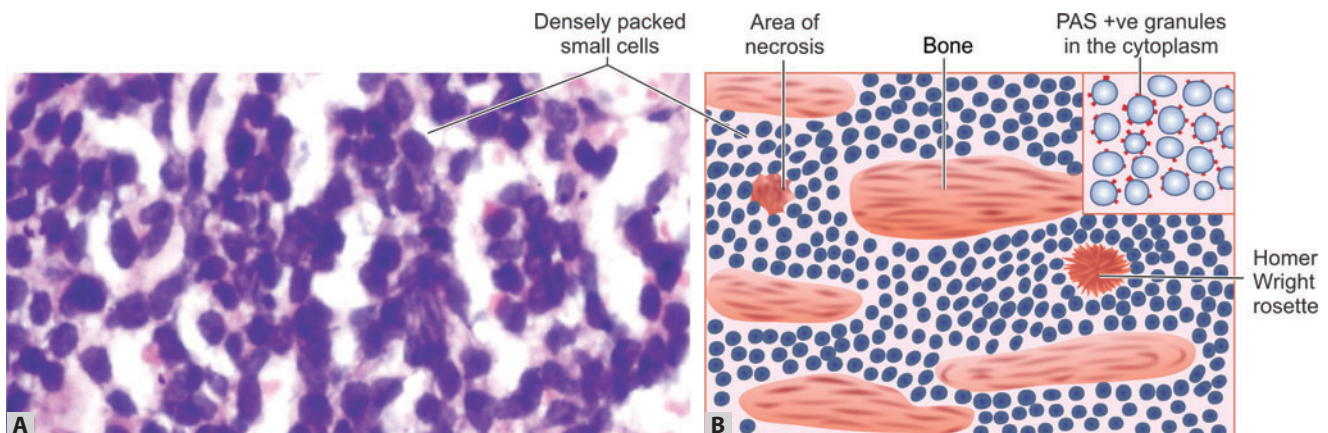
- **Local spread:** It may spread From medullary cavity **into cortex, periosteum and surrounding soft tissue**.
- **Blood spread:** Lungs, brain and other bones (skull).

Treatment includes chemotherapy and surgical excision with or without irradiation.

OSTEOARTHRITIS

Osteoarthritis: Most common degenerative joint disease mainly involving the cartilage.

Osteoarthritis (degenerative joint disease) is a **noninflammatory, slowly progressive joint disease**, mainly involving the cartilage.



Figs 27.9A and B: (A) Photomicrograph; (B) Diagrammatic; Ewing sarcoma composed of sheets of closely packed uniform small round cells with scanty cytoplasm. The inset of B shows PAS positive granules in the cytoplasm



Fig. 27.10: X-ray of Ewing sarcoma involving the diaphysis of tibia

- **Most common disease** characterized by the **progressive destruction of articular cartilage** of weight-bearing joints of **genetically susceptible older persons**.
- It leads to narrowing of joint, subchondral bone thickening, and finally **nonfunctioning, painful joint**.
- **Joints affected:**
 - **Weight bearing joints** (knee, hips and cervical and lumbar segments of the spine).
 - **Non-weight bearing proximal and distal interphalangeal joints** of the fingers, first carpometacarpal joints, and first tarsometatarsal joints of the feet.

Types

- **Idiopathic or primary osteoarthritis:** It develops as aging process and may affect few (oligoarticular) or many joints.
- **Secondary osteoarthritis:** It appears in younger individuals with predisposing condition.
 - Previous injuries to a joint.
 - Congenital deformity of a joint(s).
 - Secondary to systemic disease (e.g. diabetes, marked obesity).

Pathogenesis and Morphology (Fig. 27.11)

Osteoarthritis (OA) is a **multifactorial disease** having both genetic and environmental components.

- **Genetic factors:** Genes include those involved in prostaglandin metabolism and WNT signaling.

- **Environmental components:** The major factors are aging, biomechanical stress, obesity, muscle strength and joint stability.

Chondrocyte Injury

Aging together with genetic and biochemical factors initiate chondrocyte injury.

Changes in the Articular Cartilage

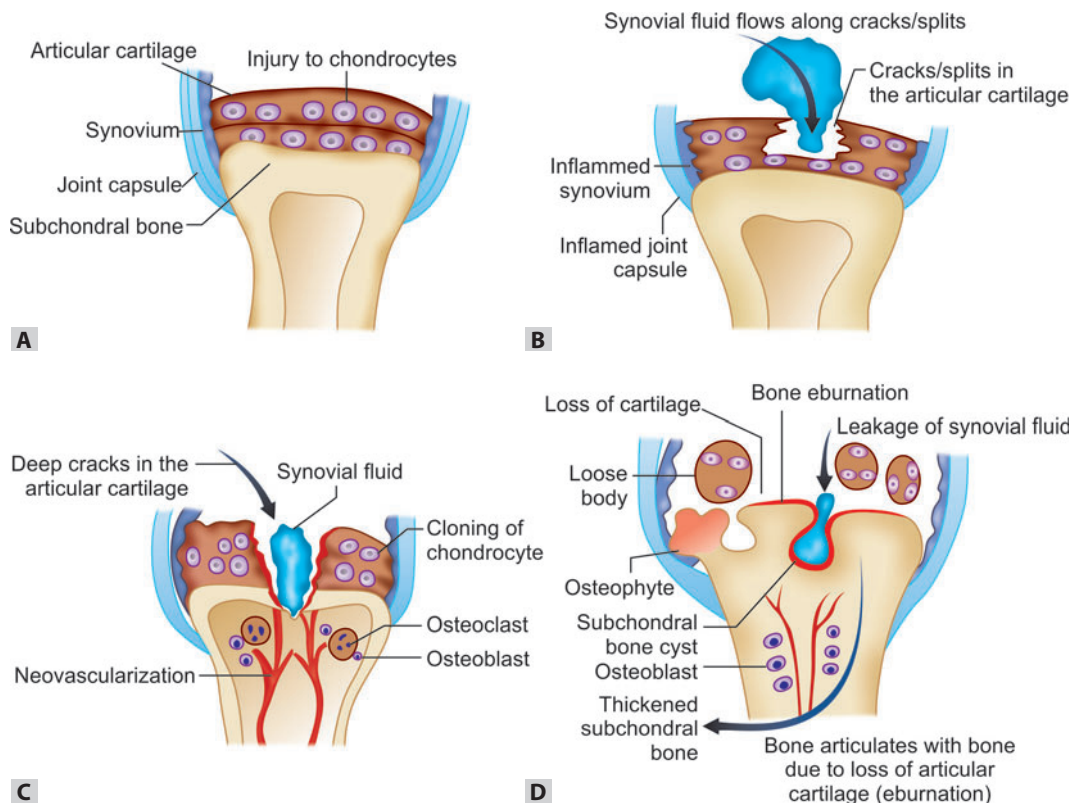
The injured chondrocytes proliferate and form clusters.

- **Cracks on superficial layers of the articular cartilage:** They develop following death of the injured chondrocytes (fibrillation).
- **Flow of synovial fluid along the cracks:** Synovial fluid penetrates deeper into the articular cartilage.
- **Inflammatory reaction:** This is initiated by inflammatory mediators secreted by injured chondrocyte. Repeated injury and chronic inflammation lead to death of chondrocytes of the articular cartilage.
- **Grossly, the articular surface of involved bone appears soft and granular.**
- **Breaking off dead pieces of articular cartilage:** It produces inflammation and foreign-body giant cell reaction in the synovium. The process may lead to sloughing of full-thickness of the cartilage. The **broken pieces of cartilage form loose bodies (joint mice)** in the synovial cavity.

Changes in the Subchondral Bone

With sloughing of the full thickness of the articular cartilage; **the subchondral bone is exposed** and becomes the new articular surface.

- **Bone eburnation:** The friction of the opposing articular surface smoothens and burnishes the exposed subchondral bone. The subchondral bone appears thick, shiny, smooth giving it the appearance of polished ivory and known as **bone eburnation** (eburnated means ivory-like). New vessels growing from the epiphysis, and fibrocartilage gets deposited.
- **Subchondral bone cyst:** The eburnated bone may crack in some areas (fracture gaps) and forces synovial fluid from the joint surface into the subchondral bone marrow regions but cannot exit. Because of this one-way ball valve-like mechanism, a **subchondral bone cyst** filled with synovial fluid is formed. The loculated fluid collection increases in size surrounded by reactive bone wall.



Figs 27.11A to D: Pathogenesis and morphological changes of osteoarthritis: (A) Aging together with genetic and biochemical factors initiate chondrocyte injury; (B) Death of chondrocytes leads to a crack in the articular cartilage and the synovial fluid flows into these cracks and leads to further loss and degeneration of cartilage; (C) Cartilage gradually worn away and new vessels grow (neovascularization) in from the epiphysis, and fibrocartilage is deposited; (D) The subchondral bone is exposed, which becomes thickened and eburnated. The broken pieces of cartilage form loose bodies (joint mice) in the synovial cavity. If there is a crack in this region, synovial fluid leaks into the marrow space and produces a subchondral bone cyst. Mushroom-shaped pearly-grayish bony spurs known as osteophytes develop at the periphery of the joint surface

- **Development of osteophytes:** Mushroom-shaped pearly grayish bony outgrowths (spurs) known as **osteophytes** develop at the periphery of the joint surface. The synovium shows mild fibrosis and chronic inflammatory cells.

Clinical Features

- Osteoarthritis is an **insidious slowly progressive disease**, and causes long-term disability.
- Present with **deep, aching pain**, which worsens with joint movement and is relieved by rest.
- Involved **joints may be swollen, tender**, and may demonstrate crepitus.
- Osteophytes in spine can cause nerve root compression and neurologic deficits.
- **Prominent osteophytes** at the **distal interphalangeal joints** are known as **Heberden nodes**, and seen commonly in women.

RHEUMATOID ARTHRITIS

Q. Write short note on pathogenesis of rheumatoid arthritis.

Rheumatoid arthritis (RA) is a **systemic, chronic inflammatory disorder**, which mainly affects the joints but can affect many other tissues and organs.

Etiology

Rheumatoid arthritis: Chronic autoimmune inflammatory disorder which mainly affects the joints.

Cause of rheumatoid arthritis remains **unknown**. Following three factors play an important role.

1. **Genetic factors:** Genetic susceptibility is a **major factor** in the pathogenesis of rheumatoid arthritis.

RA: HLA-DRB1 and polymorphism in PTPN 22.

- **HLA genes:** RA is linked to specific **HLA-DRB1 locus**.

- **Non-HLA genes:** Polymorphism in *PTPN22* gene, which encodes a tyrosine phosphatase.
- 2. **Environmental arthritogen agents:** They are thought to initiate the disease process. **Smoking** and several **microbial agents** (e.g. virus, mycobacteria and *Mycoplasma*) have been suggested but not proved.
- 3. **Autoimmunity:** The initial inflammatory synovitis, an autoimmune reaction with T cells is responsible for the chronic destructive nature of rheumatoid arthritis.

Pathogenesis (Fig. 27.12)

Rheumatoid arthritis: Loss of self-tolerance activates mainly CD4+ T cells and initiates cytokine-mediated inflammation.

- **Breakdown of self-tolerance:** Rheumatoid arthritis initiated in a **genetically susceptible individual** by an **exposure to an unknown arthritogenic antigen** → which results in a breakdown of immunological self-tolerance.

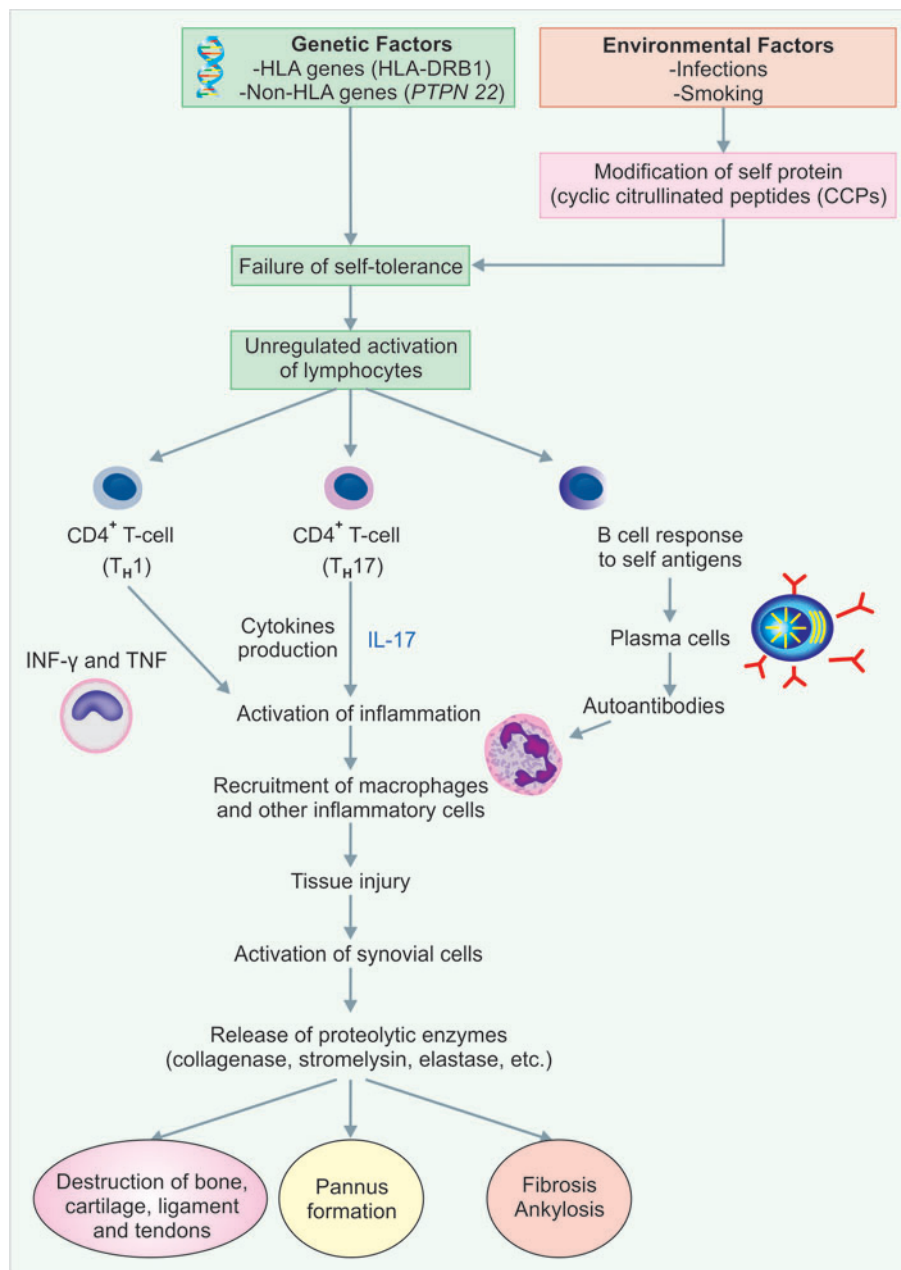


Fig. 27.12: Pathogenesis of rheumatoid arthritis

Rheumatoid arthritis: Cytokine TNF plays main role.

- **Cytokine-mediated inflammation:** Loss of self-tolerance activates mainly **CD4+ T cells** and initiates cytokine-mediated inflammation. The **principal source of cytokines is CD4+T cells** (T_H1 and T_H17). The **cytokines** (e.g. interferon- γ TNF, IL-1, IL-8, IL-6 and IL-17) **recruit macrophages and other inflammatory cells**. The products of these inflammatory cells cause tissue injury as well as activation of resident synovial cells (synoviocytes).
- **Activation of B lymphocytes:** They mainly produce two types of autoantibodies.

Rheumatoid arthritis: B cells produce rheumatoid factor, which is an IgM antibody which has specificity against Fc portion of IgG.

1. **Anti-CCPs:** Many patients have **antibodies against cyclic citrullinated peptides (CCPs)**.
 - CCPs include fibrinogen, type II collagen, α -enolase, and vimentin.
 - CCPs are derived from proteins in which arginine residues are posttranslationally converted to citrulline residues.
 - Antibodies against CCPs (anti-CCPs) form immune complexes and deposit in various tissues, main being the joints.
 - These antibodies are **useful for diagnosis** and may be involved in tissue injury.
2. **Rheumatoid factor:** About 80% of rheumatoid arthritis patients have autoantibodies called as **rheumatoid factor**.
 - **Rheumatoid factor is serum immunoglobulin M (IgM) autoantibody that binds to Fc portion of own/self IgG.**
 - Rheumatoid factor may form immune complexes with self-IgG and can produce damage to joints and other tissues.
 - Rheumatoid factor is absent in some patients with rheumatoid arthritis (seronegative).
- **Production of proteolytic enzymes:** Inflammation causes tissue injury and activation of synviocytes. Activated synoviocytes produce proteolytic enzymes, such as **collagenase, stromelysin, elastase**, etc. which **along with antigen-antibody complexes destroy the articular cartilage, ligaments and tendons of the joint**. TNF family of cytokines may cause increased activity of osteoclasts and produce destruction of bone.
- **Consequences:** The above actions bring out **edema, hyperplasia of synoviocytes and inflammatory infiltration in the synovium, which forms pannus**. The pannus adheres and grows over the articular surface causing **destruction of cartilage and erosion of adjacent subchondral bone**.

MORPHOLOGY

Joints (Fig. 27.13)

Rheumatoid arthritis causes **most severe changes in the joints**.

- **Joints involved: Symmetrical and bilateral.** Most commonly affected are **diarthrodial joints**, such as: **Proximal interphalangeal and metacarpophalangeal joints, elbows, knees, ankles, and spine**.
- **Joint lesions:** It produces a **chronic nonsuppurative proliferative polyarthritis** and inflammatory **synovitis**. As it progresses, it **destroys the articular cartilage** and causes **ankylosis of the joints**.

Synovium (Fig. 27.13)

- **Gross:** The involved synovium shows **edema, thickening, and hyperplasia of lining synoviocytes**. This results in conversion of smooth surfaced **synovium** into **delicate and bulbous papillary structures**.
- **Microscopy:** Its characteristic histologic features include:

Pannus is characteristic of: Rheumatoid arthritis.

- **Synovial hyperplasia:** The lining synovial shows 6–10 layers of synoviocytes compared to normal 2–3 layers. These hyperplastic synoviocytes **may form villus or finger-like structures**.
- **Dense inflammatory infiltrate:** Consisting of **lymphocytes** (mostly CD4+ helper T cells), **B cells, plasma cells, and macrophages**. Lymphocytes may **aggregate to form lymphoid follicles**. Neutrophils in the synovial fluid and on the synovial surface.
- **Rice bodies:** **Fibrin** covering the synovium **may float in the joint space as rice bodies**.
- **Pannus formation:** Pannus is a **mass of synovium and synovial stroma** consisting of **inflammatory cells, granulation tissue, and synovial fibroblasts**. Pannus grows over the articular cartilage and destroys it.
- **Ankylosis:** After the destruction of cartilage, the **pannus bridges the opposing bones** to form a **fibrous ankylosis**, which may ossify resulting in **bony ankylosis**. **Destruction of joint followed by fusion is termed ankylosis**.
- **Osteoporosis:** Osteoclastic activity in underlying bone cause erosions and osteoporosis of the underlying bone.

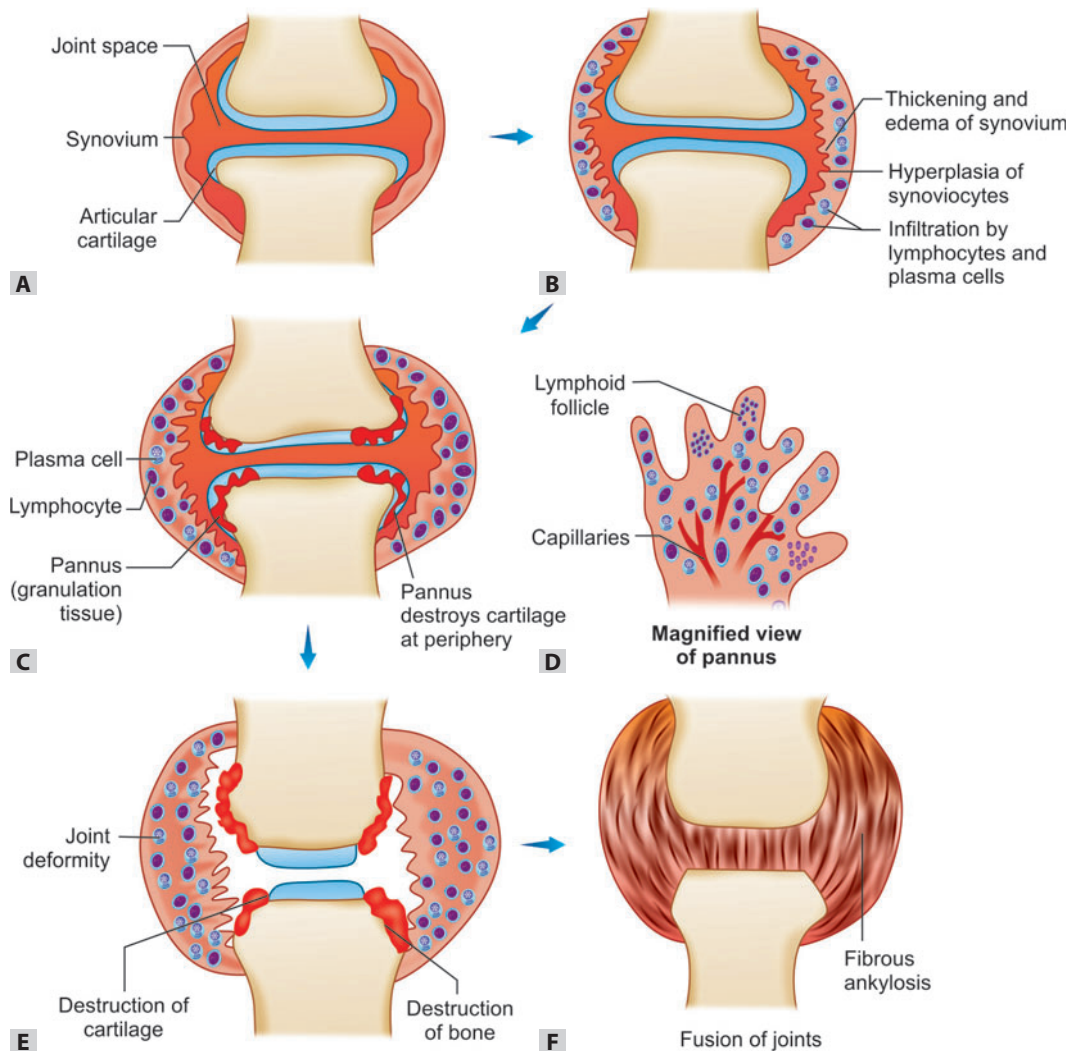
Skin

Rheumatoid nodules are the **most common cutaneous lesion**.

- **Sites:** Develops **at sites to pressure**. Example, ulnar aspect of the forearm, elbows, occiput, and lumbosacral area.
- **Gross:** **Round to oval, firm, nontender**, nodules in the subcutaneous tissue.
- **Microscopy:** It consists of a **central zone of fibrinoid necrosis surrounded by epithelioid histiocytes** (activated macrophages), **lymphocytes and plasma cells**.

Blood Vessels

Rheumatoid **vasculitis** is a dangerous complication of rheumatoid arthritis, especially when it affects vital organs. It may also affect heart, lungs, and muscles.



Figs 27.13A to F: Sequence of morphological changes in rheumatoid arthritis: (A) Normal joint; (B) Synovial cells proliferate and synovial tissue is infiltrated by lymphocytes and plasma cells; (C) The synovial hypertrophy and hyperplasia leads to formation of villi. This is accompanied by neovascularization with pannus formation (D), which covers the cartilage. The lymphoid cells form follicles; (E) Proliferating synovium extends into the joint space, destroys cartilage as well as the bone beneath the articular cartilage; (F) The joint is eventually destroyed and becomes fused, a condition termed ankylosis

Clinical Course

- **Age:** RA can occur at any age, but most common between 40 and 70 years.
- **Sex:** Three to five times more often in **women than men** (3:1).
- **Slow and insidious in onset** and presents with malaise, fatigue, and generalized musculoskeletal pain.
- **Usually small joints are involved before larger ones** and are **symmetrical**. It affects hands (metacarpophalangeal and proximal interphalangeal joints) and feet, followed by the wrists, ankles, elbows, and knees. The affected

joints are swollen, warm, painful, and stiff on arising or following inactivity.

- **Radiography:**

- Effusions into the joint.
- Narrowing of the joint space with loss of articular cartilage.
- Erosions and osteopenia of juxta-articular bone.

- **Deformities:**

- Destruction of tendons, ligaments, and joint capsules produces characteristic deformities. These consist of **radial deviation of the wrist, ulnar deviation of the**

fingers, and flexion-hyperextension abnormalities of the fingers (**swan neck**).

- Deformed joints have no stability, lose their range of motion.
- Large synovial cysts, like the **Baker cyst** may develop on posterior knee, due to increased intra-articular pressure causing outside projection of the synovium.

Laboratory Findings

Rheumatoid arthritis: Specific tests

- Rheumatoid factor
- Anti-CCP antibody.
- **Rheumatoid factor and anti-CCP antibody are specific for rheumatoid arthritis.**

GOUT AND GOUTY ARTHRITIS

Q. Write short note on gout/gouty tophi.

Gout is a **heterogeneous group of diseases** characterized by **hyperuricemia** (plasma urate level above 6.8 mg/dL) and **urate crystal deposition in joints and kidneys**.

Classification (Box 27.1)

Depending on the etiology of the hyperuricemia.

- **Primary (idiopathic):** Hyperuricemia occurs without any other disease.
- **Secondary:** Hyperuricemia occurs in association with another illness.

Etiology

- Uric acid is the end product of purine metabolism, which is eliminated only in the urine. Humans do not have uricase, an enzyme which degrades uric acid. Usually,

BOX 27.1: Classification of gout

Primary gout (90%)

- Increased production of uric acid
 - Increased dietary intake of purine
 - Due to inborn errors of purine metabolism
- Decreased excretion of uric acid with normal production

Secondary gout (10%)

- Increased production of uric acid with increased urinary excretion
 - Increased catabolism of nucleic acid due to increased cell turnover (e.g. leukemias, cancers, and during tumor lysis induced by chemotherapy)
- Decreased excretion of uric acid with normal production
 - Chronic renal disease

there is a balance between uric acid production and tissue deposition of urates.

- **Hyperuricemia** is necessary, but not sufficient for the development of gout. Several factors, which convert asymptomatic hyperuricemia into primary gout. These are as follows:

- **Age:** Gout usually presents after 30 years of age.
- **Duration of the hyperuricemia:** Usually not less than 20–30 years.
- **Genetic predisposition:** Primary gout is of multifactorial inheritance and runs in families.
- **Heavy alcohol intake.**
- **Obesity.**
- **Drugs** (e.g. thiazides) which reduce excretion of urate.
- **Lead toxicity.**

Pathogenesis

Q. Write short essay/note on pathogenesis of gouty arthritis.

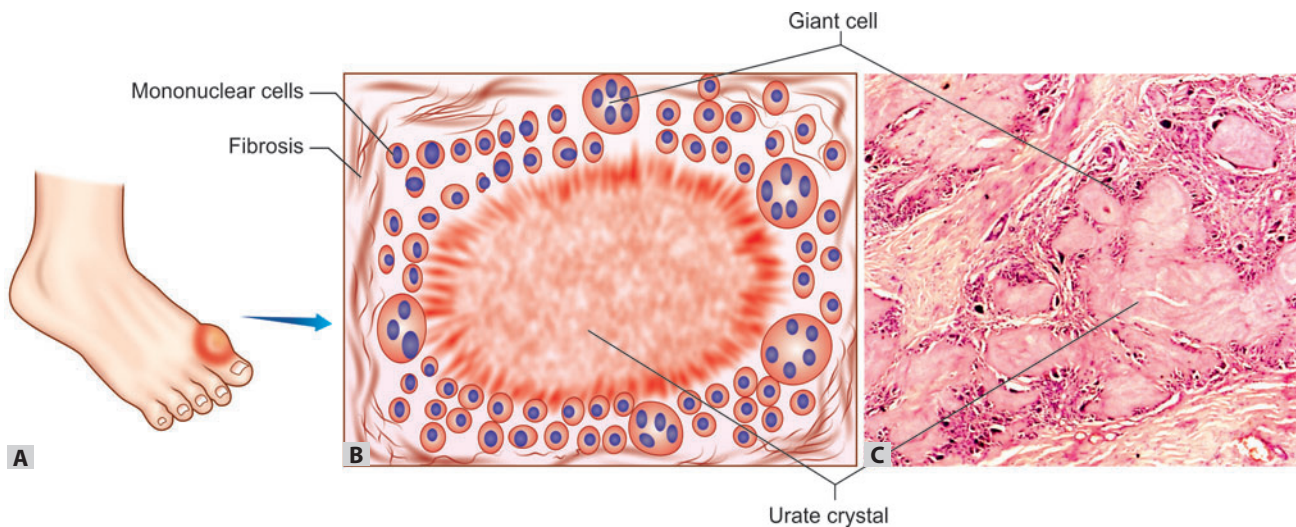
- **Precipitation of monosodium urate (MSU)** crystals into the joints.
 - The solubility of urate in a joint is dependent on intra-articular concentration of urate and the temperature (less soluble in low temperature).
 - Monosodium urate is less soluble in synovial fluid compared to plasma. So, they become easily supersaturated in the peripheral joints (ankles and toes), where temperatures are usually low.
 - With hyperuricemia, precipitation of urate crystals develops in the synovium and in the joint cartilage.

Acute Arthritis

- Probably due to trauma, the precipitated crystals are released into the synovial fluid, which results in inflammatory response, which is characteristic of the acute attack.
- MSU crystals are phagocytosed by macrophages and release many cytokines (e.g. IL-1 β , IL-18).
- IL-1 β stimulates the expression of adhesion molecules causing accumulation of neutrophils at the site of acute inflammation.
- The neutrophils release toxic-free radicals, leukotrienes (leukotriene B₄), and lysosomal enzymes which aggravate inflammation and results in acute arthritis.
- Acute arthritis usually remits in days to weeks.

Chronic Arthritis

- Repeated attacks of acute arthritis lead to chronic arthritis and tophi formation in the synovial membranes and periarticular tissue.



Figs 27.14A to C: (A) Usual site of gouty tophus (diagrammatic); (B) Diagrammatic; (C) Photomicrograph; microscopy of a gouty tophus showing aggregates of dissolved urate crystals surrounded by fibroblasts, mononuclear inflammatory cells, and giant cells

MORPHOLOGY (FIG. 27.14)

The morphological changes in gout are as follows:

1. Acute arthritis

- **Acute inflammation:** It is characterized by edema, congestion and dense infiltration of synovium by neutrophils. Few lymphocytes, plasma cells, and macrophages may also be seen.
- **Monosodium urate (MSU) crystals**
 - They are long, slender, and needle shaped.
 - They are found in the cytoplasm of the neutrophils and are arranged in small clusters in the synovium.
 - As the acute attack subsides, they may be resolvable.

2. Chronic tophaceous arthritis

Tophi: Pathognomonic of gout.

- It follows the repeated acute attacks.
- The urates may heavily encrust the articular surfaces.
- The synovium shows fibrosis, thickening, and infiltration by inflammatory cells. It forms a pannus and destroys the cartilage, and juxta-articular bone erosions.
- When severe, fibrous or bony ankylosis may lead to loss of joint function.
- **Tophi** (Fig. 27.14): Lesions consisting of large aggregates of urate crystals, surrounded by inflammatory reaction are known as tophi and are the pathognomonic of gout.

- **Sites:** Tophi are commonly found in the articular cartilage of joints and in the periarticular ligaments, tendons, and soft tissues, Achilles tendons, and earlobes.
- **Microscopy:** They consist of aggregations of urate crystals surrounded by macrophages, lymphocytes, and foreign-body giant cells.

Clinical Course

Gout: Increased levels of uric acid (hyperuricemia) and urate crystal deposition in joints and kidneys.

The gout has four stages:

1. **Asymptomatic hyperuricemia:** Appears at puberty in males and after menopause in females.
2. **Acute gouty arthritis:** It appears as sudden excruciating joint pain with tenderness. Even if not treated, acute attacks of gout are usually self-limited.
3. **Intercritical gout:** The intercritical period is the asymptomatic interval between the first acute attack and subsequent attacks.
4. **Chronic tophaceous gout:** After several years (about 12 years) of acute gouty arthritis, chronic tophaceous gout may develop, which may lead to severe crippling disease.

Prognosis: Gout does not reduce the life span, but may cause morbidity.

Central Nervous System Disorders

CEREBROVASCULAR DISEASES

Definition: Cerebrovascular disease is the term for **group of disorders of the brain** caused by **pathological process** involving the blood vessels of the brain.

Pathogenic Mechanism

- **Thrombosis and embolism** → occlusion of blood vessel → infarction or ischemia injury.
- **Rupture of blood vessel** → hemorrhage → direct damage to parenchyma.

Stroke is characterized by sudden onset and cause neurologic damage due to focal ischemia or hemorrhage.

Effects of Cerebrovascular Diseases

1) Hypoxia, ischemia, and infarction or 2) hemorrhage (refer intracranial hemorrhage page no. 764).

1. **Global cerebral ischemia** (diffuse ischemic/hypoxic encephalopathy)
 - Ischemic damage occurs in the entire brain.
 - **Causes:** Generalized reduction of oxygenated blood to the brain as in shock (hemorrhagic shock), severe systemic hypotension (e.g. cardiac arrhythmia, myocardial infarction or cardiac arrest).
 - **Clinical outcome:**
 - **Mild:** Transient ischemia → confusion state followed by complete recovery.
 - **Severe:** Ischemia → results in death of neurons and the patient (if survives) may remain in a vegetative state.
2. **Focal cerebral ischemia: It may cause infarction.** Majority are caused by thrombosis and embolism.

- **Thrombosis: Atherosclerosis** → predisposes to thrombus formation → occlude the vascular lumen.
- **Embolic arterial occlusion:** More common. The origin of emboli may be:
 - **Heart:** Predisposing factors are myocardial infarct, valvular disease, and atrial fibrillation.
 - **Arteries:** For example, atheromatous plaques within the carotid arteries
 - **Other sources of emboli:** Paradoxical emboli (e.g. in children with cardiac anomalies), emboli following cardiac surgery, and other emboli like tumor, fat, or air.
- **Generalized arterial disease: Infectious vasculitis** (syphilis and tuberculosis), polyarteritis nodosa and other non-infections vasculitides.

Classification of Infarcts

1. **Hemorrhagic (red) infarction:** It is characterized by multiple, petechial hemorrhages and is seen with **embolic events**.
2. **Nonhemorrhagic (pale, bland, anemic) infarcts:** It is usually associated with **thrombosis**.

Microscopy: It shows liquefactive necrosis.

Clinical Features

- Defects associated with infarction depend on the region of brain involved rather than the cause.
- Neurologic symptoms referable to the area of injury usually develop rapidly, over minutes, and may continue to evolve over hours.

INTRACRANIAL HEMORRHAGE

Hemorrhages can occur at any site within the CNS. Depending on the anatomic location they are subdivided into:

1. **Epidural hemorrhage**] Usually associated with
2. **Subdural hemorrhage**] trauma
3. **Subarachnoid hemorrhage**: It is usually due to ruptured aneurysm, vascular malformations.
4. **Intraparenchymal hemorrhage**: It is usually spontaneous (nontraumatic) due to rupture of intraparenchymal vessel (e.g. hypertension and cerebral amyloid angiopathy).

Epidural Hemorrhage

Definition: Accumulation of blood between skull bone and the dura matter.

Causes: 1) Road accidents, 2) fracture of skull bones, and 3) fall.

Epidural hemorrhage: Fracture of skull and tear in the middle meningeal artery.

Subdural Hemorrhage

Definition: Subdural hematoma is defined as accumulation of blood in the subdural space, i.e. between the inner surface of the dura mater and the outer arachnoid layer of the leptomeninges.

Causes: It is usually due to tear in the veins at the point where they penetrate the dura matter. The causes may be: 1) fall, 2) road traffic accidents, 3) assaults, and 4) sport accidents.

Subdural hemorrhage: Venous bleeding between dura and arachnoid matter.

Subdural hemorrhage: Most commonly occurs due to rupture of bridging veins.

Subarachnoid Hemorrhage and Ruptured Saccular Aneurysms

Definition: Accumulation of blood in the subarachnoid space, i.e. between the inner surface of the arachnoid and pia matter.

Subarachnoid hemorrhage: Rupture of congenital berry aneurysm.

Q. Write short note on berry aneurysm.

Causes

- Rupture of a saccular (berry) aneurysm
- Trauma
- Rupture of a hypertensive intracerebral hemorrhage into the ventricular system
- Hematologic disorders
- Vascular malformation
- Tumors.

Saccular Aneurysms

- It is one of the cause of subarachnoid hemorrhage.

Pathogenesis

Berry aneurysm is associated with adult polycystic kidney (ADPKD).

It is found in association with polycystic kidney disease. There is congenital defect in the smooth muscle of the tunica media at the site of arterial bifurcation, where local hemodynamic factors act to produce a slowly enlarging saccular aneurysm. Common sites of berry aneurysm are shown in Figure 28.1.

MORPHOLOGY

- **Gross:**
 - **Size:** Few millimeters to 2 or 3 cm in diameter.
 - **Appearance:** Bright red, shiny surface and thin translucent wall.
 - **Location:** At an arterial branch point along the circle of Willis.
- **Microscopy:** Absence of internal elastic lamina in the neck of the aneurysm.

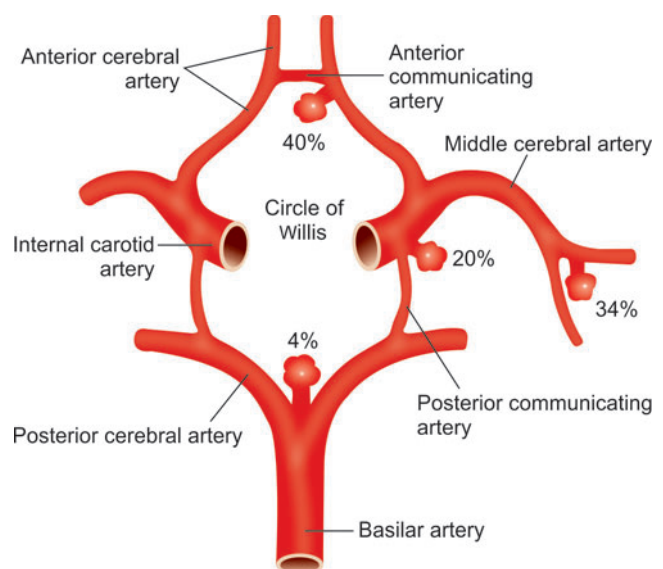


Fig. 28.1: Blood supply to the brain and circle of Willis with common sites of saccular (berry) aneurysms

Clinical Features

- Most frequent in the **fifth decade** and is slightly **more frequent in females**.
- Presents with sudden, severe headache followed by loss of consciousness.

Intracerebral (Intraparenchymal) Hemorrhage (Fig. 28.2)

Intracerebral hemorrhage: Complication of hypertension.

Definition: Hemorrhages within the brain parenchyma. Usually spontaneous and occur in middle to late adult life (peak during 60 years of age).

Etiology: Most are caused by **rupture of a small intraparenchymal vessel**. The two major causes are **hypertension** and **cerebral amyloid angiopathy (CAA)**.

Blood vessel changes in hypertension:

- Atherosclerosis in larger arteries.
- Hyaline arteriolosclerosis in smaller vessels → weaken the vessels → more vulnerable to rupture.
- Malignant hypertension → proliferative changes and frank fibrinoid necrosis of arterioles.

MENINGITIS

Q. Write short note on meningitis.

Meninges have three layers and from inside out are pia, arachnoid and dura mater.

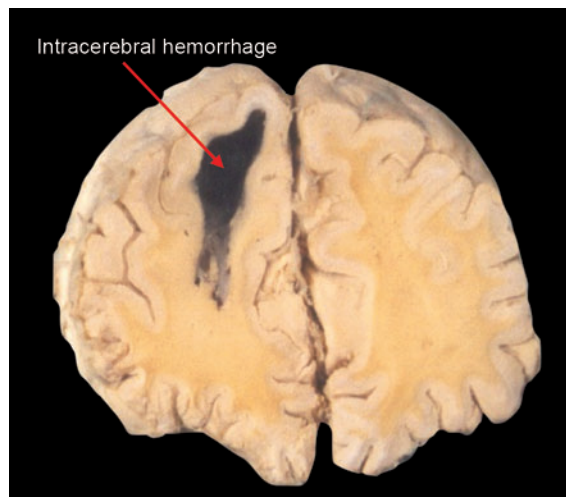


Fig. 28.2: Intraparenchymal hemorrhage on the left cerebral hemisphere appears as dark colored area

Leptomeningitis: Meningitis is the term usually used for inflammation **within subarachnoid space between pia and arachnoid matter** and known as leptomeningitis.

Meningitis is a **dangerous infection** caused by a variety of microorganisms and CSF acts an excellent culture medium for most of these causative organisms.

Meningitis: Inflammation of pia and arachnoid.

Causes

Q. Write short note on acute pyogenic meningitis.

- **Infectious meningitis:**
 - **Acute:** (1) Pyogenic (usually bacterial), (2) aseptic (usually viral).
 - **Chronic:** (1) Tuberculous, (2) syphilitic and (3) cryptococcal.
- **Chemical meningitis:** Due to a nonbacterial irritant introduced into the subarachnoid space.

Acute Pyogenic (Bacterial/Purulent) Meningitis

Etiology

- **Neonates:** *Escherichia coli* and the **group B streptococci**
- **Infants and children:** *Haemophilus influenzae*
- **Adolescents and in young adults:** *Meningococcus (Neisseria meningitidis)* is the most common pathogen.
- **Extremes of life:** *Streptococcus pneumoniae* and *Listeria monocytogenes*.

Meningitis: Different pathogens can reach through different routes.

Routes of infection: Infectious microbes may enter the meninges by following routes.

- **Hematogenous route**
- From an **adjacent focus of infection**
- **Iatrogenic:** Through lumbar puncture.

MORPHOLOGY

Gross

Thick creamy exudate is seen within the leptomeninges **over the surface of the brain** (cerebral hemispheres). The meninges appear **opaque**.

Location of the exudate

- *H. influenzae* meningitis—usually basal.
- Pneumococcal meningitis—over the cerebral convexities near the sagittal sinus.

Microscopy

- Exudates in the subarachnoid space: Consists of dense collections of neutrophils and fibrin
- **Gram stain** may reveal the causative organism.

CSF Changes

Q. Write short note on CSF changes in pyogenic meningitis.

Presence of polymorphonuclear leukocytes in the CSF is the most definitive feature of meningitis.

- **Appearance:** Normally CSF is clear. In acute meningitis, it becomes **cloudy or frankly purulent**.
- **Pressure: Increased** (>180 mm of water).
- **Cells: Increased neutrophils** (may be as many as 90,000 per cubic millimeter).
- **Protein concentration: Increased** (>50 mg/dL).
- **Glucose: Markedly reduced** (<40 mg/dL).
- **Smear: Bacteria** may be seen on a smear (Gram stain) or can be cultured.

CSF in acute pyogenic meningitis:

- Cloudy
- Proteins increased
- Sugar decreased
- Numerous polymorphonuclear leukocytes.

Complications of Bacterial Meningitis

- **Obstructive hydrocephalus**
- **Thrombophlebitis of leptomeningeal veins** may lead to
 - Venous thrombosis
 - Cerebral infarction
 - Focal infection of the underlying brain parenchyma.
- **Chronic adhesive arachnoiditis**
- **Cerebral abscess**
- **Subdural empyema**
- **Epilepsy**
- **Waterhouse-Friderichsen syndrome:** It results from meningitis-associated septicemia with hemorrhagic infarction of the adrenal glands and cutaneous petechiae. It occurs most often with meningococcal and pneumococcal meningitis.

Waterhouse-Friderichsen syndrome:

- Meningitis
- Septicemia
- Hemorrhagic infarction of the adrenal glands
- Cutaneous petechiae.

Clinical Features

Bacterial meningitis: Majority of organisms originate in nasopharynx.

- **Symptoms: Headache, vomiting, fever and convulsions** (especially in children).

- Classic signs of meningitis
 - **Cervical rigidity/neck stiffness**
 - **Kernig sign: Knee pain with hip flexion**
 - **Brudzinski sign: Knee/hip flexion when the neck is flexed.**

Untreated pyogenic meningitis can be fatal.

Acute Aseptic (Viral) Meningitis

Viral meningitis: Most often transmitted by fecal-oral route.

Symptoms of acute meningitis with **no identifiable organisms**.

Etiology: Enterovirus, HIV, mumps virus, EB virus and herpes simplex virus.

Age: Common in children and young adults.

CSF Findings

- **Appearance:** Clear.
- **Protein:** Moderately increased (>40 mg/100 mL).
- **Glucose:** Always normal.
- **Microscopy:** 10 to 100 lymphocytes/mL.

Clinical features: Fever and headache.

Prognosis: Usually self-limiting.

CSF findings in various types of meningitis are summarized in Table 28.1.

Chronic Meningitis

Types

- **Bacterial:** 1) Tuberculous caused by *M. tuberculosis* and 2) syphilitic caused by *T. pallidum*.
- **Fungal:** Cryptococcal.

Tuberculous Meningitis

Q. Write short note on tuberculous meningitis.

Infection of the meninges by tubercle bacilli.

Etiology: *Mycobacterium tuberculosis* human type is the most common cause.

Mode of Infection

- **Hematogenous route** from other site (most commonly from lung)
- **Miliary spread**
- **Direct spread** from adjacent site such as vertebral body.

TABLE 28.1: Cerebrospinal fluid findings in meningitis

	<i>Normal</i>	<i>Acute pyogenic</i>	<i>Acute viral</i>	<i>Tuberculous</i>
Physical examination	Clear and colorless	Turbid and forms coagulum	Clear	Clear and colorless, forms cobweb on standing due to coagulation of fibrinogen
CSF pressure	60–150 mm of H ₂ O	Raised above 180 mm of H ₂ O	Raised above 250 mm of H ₂ O	Raised above 300 mm of H ₂ O
Total protein	20–40 mg/100 mL	50–200 mg/100 mL	>40 mg/100 mL	50–150 mg/100 mL
Glucose	45–80 mg/100 mL	0–20 mg/100 mL	Normal	20–50 mg/100 mL
Chlorides	720–750 mg/100 mL	600–700 mg/100 mL	Normal	450–600 mg/100 mL
Cells				
Polymorphs	Usually absent	150–2000/μL	Absent	0–5 cells/μL
Lymphocytes	0–5 cells/μL	5–50 cells/μL	10–100 cells/μL	500–700 cells/μL
Gram stain/ZN stain	-	Bacteria +	-	AFB +

MORPHOLOGY**Gross**

Tuberculous meningitis:

- Greenish, gelatinous or fibrinous exudate
- Most prominent at the base of the brain surrounding cranial nerves.
- **Subarachnoid space** contains a **greenish, gelatinous or fibrinous exudate**, most prominent at the base of the brain surrounding cranial nerves.
- Leptomeninges may show discrete, white granules of tubercle.

Microscopy

- **Granuloma** consisting of epithelioid cells, Langhans giant cells surrounded by lymphocytes. It may show central area of caseous necrosis
- AFB stain may show acid fast bacilli.

Clinical Features

Includes headache, malaise, mental confusion, and vomiting. On examination, there will be neck rigidity.

CSF**Q. Write short note on CSF changes in tuberculous meningitis.**

- **Physical examination:** Clear and colorless, forms cobweb on standing due to coagulation of fibrinogen.
- **CSF pressure:** Raised above 300 mm of H₂O
- **Protein:** Raised ranges from 50–150 mg/100 mL
- **Glucose:** Moderately reduced or normal (20–50 mg/100 mL)
- **Chloride:** Decreased (450–600 mg/100 mL)
- **Cells:** Moderate CSF pleocytosis (500–700 cells/μL), mainly lymphocytes.

Complications: 1) Hydrocephalus, 2) nerve root damage, and 3) tuberculous encephalitis.

CSF in tuberculous meningitis:

- Forms cobweb on standing
- Proteins increased
- Sugar decreased
- Chlorides decreased
- Lymphocytes.

Meningitis: Neck rigidity.

Tuberculoma

It is another manifestation of tuberculosis of CNS.

MORPHOLOGY**Gross:**

- **Number:** Single or multiple well circumscribed intraparenchymal mass (tuberculoma).
- **Size:** Varies and may be as large as several centimeters in diameter, and may present as intracranial space occupying lesion.

Microscopy: Shows **epithelioid granuloma** consisting of central core of caseous necrosis surrounded by epithelioid cells, Langhans giant cell and lymphocytes.

Neurosyphilis

Develops in the tertiary stage of syphilis and occurs in about 10% of untreated patients.

Major Patterns of CNS Involvement

1. **Meningovascular neurosyphilis:** Chronic meningitis.
 - Involves the base of the brain and may also involve the cerebral convexities and the spinal leptomeninges.

- Obliterative endarteritis (Heubner arteritis) Characterized by a distinctive perivascular inflammatory reaction rich in plasma cells and lymphocytes.
 - Cerebral gumma is mass rich in plasma cell seen in parenchyma and may also be seen in the meninges.
2. **General paresis of the insane:** Due to invasion of the brain by *Treponema pallidum*
- Insidious in onset but progressive mental deficits with mood alterations that terminate in severe dementia (**general paresis of the insane**).
- **Microscopy:** 1) Loss of neurons, 2) proliferations of microglia (rod cells); and 3) gliosis. Iron deposits mainly in the perivascular region and in the neurophil, which stain positive with the Prussian blue stain.
3. **Tabes dorsalis:** Due to damage to the sensory nerves in the dorsal roots.
- Clinical features:
 - Impaired joint position sense and resultant ataxia (locomotor ataxia).
 - Loss of pain sensation, leading to skin and joint damage (Charcot joints).
 - Other sensory disturbances: Lightning pains and absence of deep tendon reflexes.
 - **Microscopy:** Loss of both axons and myelin in the dorsal roots resulting in atrophy of dorsal columns of the spinal cord.

TUMORS OF CNS

Classifications: Important CNS tumors include

1. **Gliomas:** Astrocytomas, oligodendroglioma, ependymoma.
2. **Embryonal (primitive) neoplasms:** Medulloblastoma.
3. **Neuronal tumors:** Central neurocytomas, ganglioglioma.
4. **Meningiomas.**
5. **Metastatic tumors.**

GLIOMAS

Gliomas are the **most common group of primary brain tumors**. The tumors are classified histologically on the resemblance of cells to glial cells.

Major tumors includes: 1) astrocytomas 2) oligodendrogliomas and 3) ependymomas.

Gliomas: Most common primary brain tumors includes:

1. Astrocytomas
2. Oligodendrogliomas
3. Ependymomas.

Astrocytoma

Q. Write short note on astrocytoma.

Astrocytoma: Most common primary brain tumors in adults.

- Astrocytoma is a **glioma derived from astrocytes**.
- Subclassification: Two major categories
 - Diffusely infiltrating astrocytomas
 - **Localized astrocytomas:** Pilocytic astrocytomas.

Diffusely Infiltrating Astrocytomas

- Form—**80% of primary brain tumors in adults**.
- **Site:** Usually in the **cerebral hemispheres**. Other sites include: cerebellum, brainstem, and spinal cord.
- **Age:** Usually **fourth to sixth decades**.
- **Classification:** Diffuse astrocytomas can be further categorized according to their histologic differentiation and clinical course into:
 - **Diffuse astrocytoma (grade II)**
 - **Anaplastic astrocytoma (grade III)**
 - **Glioblastoma (grade IV)**.

There are **no WHO grade I** infiltrating astrocytomas.

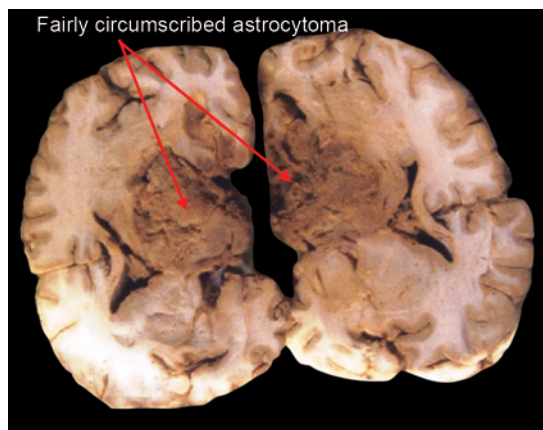
Molecular Genetics

Before modern advances in genetic analyses, glioblastoma were divided into—

1. **Primary glioblastoma:** It arises **de novo** as new onset disease, without any pre-existing low-grade astrocytoma and occurs at older age.
2. **Secondary glioblastoma:** It arises **in patient who had lower-grade astrocytoma earlier** and occur in younger patients.

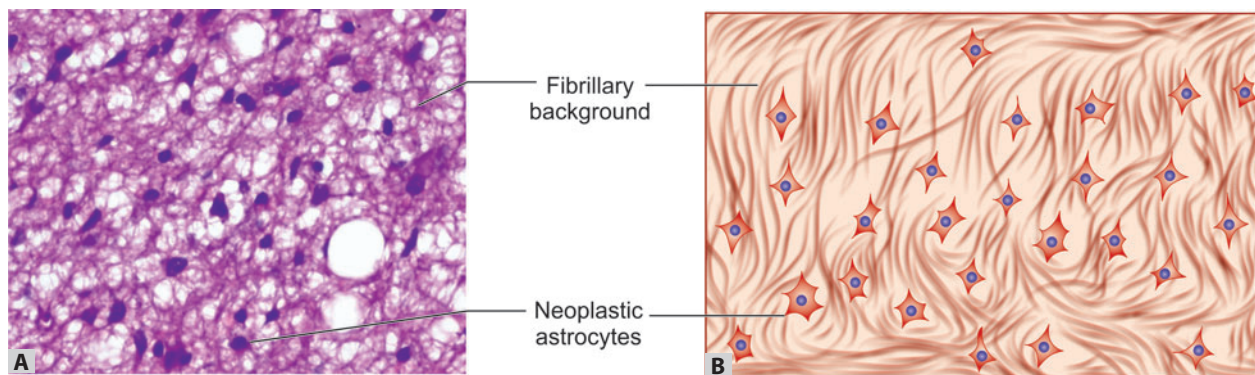
Molecular subtypes: According to patterns of molecular alteration in glioblastoma, it is divided into four molecular subtypes: classic, proneural, neural, and mesenchymal.

1. **Classic subtype:** Forms the major subtype of primary glioblastoma. It is characterized by 1) mutations of the PTEN tumor suppressor gene, 2) deletions of chromosome 10, and 3) amplification of the EGFR oncogene. Other molecular changes include focal deletions involving chromosome 9p21→producing hemizygous deletion of the CDKN2A tumor suppressor gene.
2. **Proneural subtype:** Most common type associated with secondary glioblastoma. It is characterized by 1) mutations of TP53, and 2) point mutations in the isocitrate dehydrogenase genes, IDH1 and IDH2. It often shows an overexpression of the receptor for platelet-derived growth factor receptor α (PDGFRA).


Glioblastoma:

- Increased cellularity
- Pleomorphism
- Serpentine necrosis
- Pseudopalisading
- Endothelial cell proliferation.

Fig. 28.3: Astrocytoma involving cerebrum



Figs 28.4A and B: Microscopic appearance of diffuse astrocytoma. (A) Photomicrograph; (B) Diagrammatic

3. **Neural subtype:** It is characterized by higher levels of expression of neuronal markers, such as NEFL, GABRA1, SYT1, and SLC12A5.
4. **Mesenchymal subtype:** It is characterized by deletions of the NF1 gene on chromosome 17, and lower expression of the NF1 protein. There is high expression of genes involved in the TNF pathway and the NF- κ B pathway.

Common features of different molecular subtypes is they mostly affect two cancer hallmarks namely 1) **sustained proliferative signaling** and 2) **evasion of growth suppressors**. For example, in proneural glioblastoma, there is overexpression of PDGFRA and in classic glioblastoma mutation, there is amplification of EGFR genes. Both these molecular changes causes increased receptor tyrosine kinase signaling \rightarrow which in turn stimulate RAS and PI3K/AKT signaling \rightarrow leads to activation of cells from the G1 to S phase of the cell cycle \rightarrow tumor growth. Other molecular events may directly or indirectly inhibit RB and p53 function. Mutations that activate RAS and PI-3 kinase and inactivate p53 and RB are probably occurs in 80–90% of primary glioblastomas. In higher grade astrocytomas (WHO grades III and IV), the presence of the mutant form of *IDH1* (mainly the R132H mutation) is associated with better prognosis. *IDH1* mutations causes activation of neomorphic enzyme which

stimulate oncogenesis by inhibiting enzymes that regulate DNA methylation (epigenetic dysregulation).

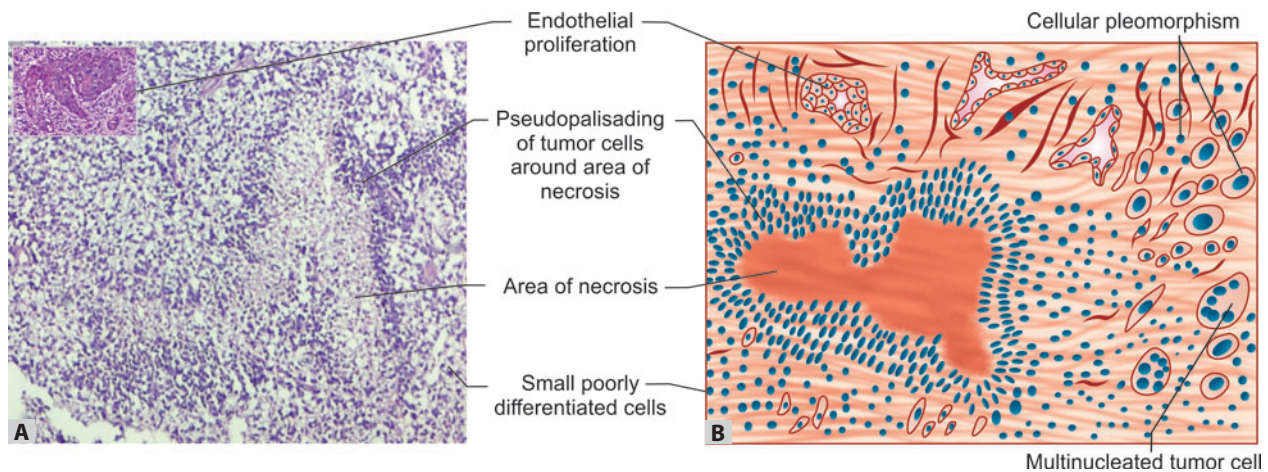
MORPHOLOGY

Diffuse astrocytoma

- **Gross** (Fig. 28.3):
 - **Poorly demarcated, infiltrative tumor**
 - **Size:** Range from a few centimeters to large lesions.
 - **Cut surface:** **Gray, firm or soft and gelatinous.** May show cystic degeneration.
- **Microscopy** (Fig. 28.4):
 - **Mild to moderate cellularity** due to increase in the astrocytic glial tumor cells.
 - Variable degree of nuclear pleomorphism.
 - **Fibrillary background:** Between the nuclei of tumor cell, extensive feltwork of fine, GFAP-positive astrocytic processes produces a fibrillary background appearance. The demarcation between neoplastic and normal tissue is indistinct, and tumor cells infiltrate surrounding normal tissue some distance away from the main tumor.
- **Immunohistochemistry:** These glial neoplasms show immunopositivity for GFAP (glial fibrillary acid protein).

Anaplastic astrocytomas (grade III)

- Increased cellularity



Figs 28.5A and B: Microscopic features of glioblastoma (A) Photomicrograph; (B) Diagrammatic. Foci of necrosis with pseudopalisading of tumor nuclei and endothelial cell proliferation are seen. Inset in A shows vascular endothelial proliferation

- Cellular and nuclear pleomorphism
- Anaplasia
- Presence of mitotic figures
- Rapid growth of the tumor.

Glioblastoma

Q. Write short note on glioblastoma.

Glioblastoma: Previously known as glioblastoma multiforme (GBM), because of variation in the gross appearance of the tumor from region to region.

- **Gross:**
 - Consistency and color varies.
 - **Variegated appearance:** Some areas are firm and white, others are soft and yellow due to necrosis.
 - Colors represent multiple areas of **recent (red) and old (yellow) hemorrhage.**
 - Show cystic degeneration.
- **Microscopy (Fig. 28.5): Similar to anaplastic astrocytoma with necrosis or vascular/endothelial cell proliferation.**
 - Increased cellularity
 - Marked cellular and nuclear pleomorphism
 - Anaplasia
 - Frequent mitoses
 - **Necrosis: (1) Serpentine (snake like) pattern of necrosis and (2) edges of the necrotic area is surrounded by the palisading tumor cells, which is known as pseudo-palisading (garlanding).**
 - **Vascular or endothelial cell proliferation:** It is characterized by **tufts of piled-up endothelial cells** (at least two layers), which **protrude into the lumen**. Marked endothelial cell proliferation may form structure similar to glomerulus and known as glomeruloid body.
- **Gliomatosis cerebri:** It is a diffuse glioma showing extensive infiltration of multiple regions of the brain (or the entire brain). It is aggressive and a grade III/IV lesion.

Clinical features: Seizures and headaches. Symptoms depend on the location and growth rate of the tumor. Well-differentiated diffuse astrocytomas may remain stable or progress slowly. Clinical deterioration invariably occurs due to the more rapid growth and higher histological grade.

Glioblastoma multiforme: High-grade astrocytoma with worst prognosis.

Radiologic Studies

Show mass effect and **edema of brain adjacent to the tumor**. **High-grade astrocytomas** have abnormal vessels that are “leaky” and demonstrate **contrast enhancement** on imaging studies.

Prognosis: Diffuse astrocytomas may remain static or progress only slowly over a number of years. **Glioblastoma** has a **very poor prognosis**.

Pilocytic Astrocytoma (Grade I/IV)

Pilocytic astrocytomas are **relatively benign (grade I/IV)**, grow very slowly and have an **excellent prognosis**. They are **distinguished from the other types of astrocytoma by their gross and microscopic appearance**. Unlike other astrocytomas, *TP53* mutations rare.

Age: Occur in **children and young adults**.

Site: Usually located in the **cerebellum, floor and walls of the third ventricle** and optic nerves.

MORPHOLOGY

Gross

Usually **cystic or solid** and **well circumscribed**.

Microscopy

Usually shows **biphasic pattern**.

- **Fibrillary areas:** They are composed of **bipolar astrocytes with abundant, long, thin hair-like glial processes**, which form dense fibrillary meshworks. These **processes are GFAP-positive**.
- **Loose microcystic areas**
- Cells typically contain **Rosenthal fibers** and eosinophilic granular bodies.
- **Do not show infiltration** into the surrounding brain.

Oligodendroglioma (WHO Grade II/IV)

Q. Write short note on oligodendroglioma

- Constitute 5–15% of gliomas. This is an infiltrating gliomas composed of tumor cells that resemble oligodendrocytes.
- **Age:** Most common during **fourth and fifth decades**.

Molecular Genetics

Most common genetic alterations are mutations of the isocitrate dehydrogenase a gene (*IDH1* and *IDH2*) observed in about 90% of cases and has a better prognosis. Loss of 9p, loss of 10q, and mutations in *CDKN2A* occur with progression to anaplastic oligodendroglioma. Tumors with co-deletion of 1p/19q (in ~ 80% of cases) respond well to chemotherapy and radiation, and those without loss of 1p or 19q appear to be resistant to chemotherapy regimens.

MORPHOLOGY

Oligodendroglioma:

3 Cs

- Clear halo of cytoplasm (fried egg appearance) in the tumor cells
- Calcification
- Chicken-wire appearance of anastomosing capillaries.

Gross

- **Site:** Common in the **white matter of the cerebral hemispheres**
- **Well-circumscribed, gelatinous**, and gray
- May show **cysts, areas of hemorrhage**, and **calcification**.

Microscopy (Fig. 28.6)

- Sheets of **small, round, and regular cells**.
- **Nuclei:** **Spherical nuclei with fine granular chromatin**
- **Cytoplasm:** Nuclei are surrounded by a **clear halo of cytoplasm** giving rise to **fried egg appearance** to the cell.
- **Stroma:** It consists of **delicate network of anastomosing capillaries (chicken-wire appearance)**.
- **Calcification** (calcospherites) is common.
- Mitotic figure is usually not seen.

Prognosis: Better than astrocytomas.

Oligodendroglioma: Deletion of chromosome 1p and 19q.

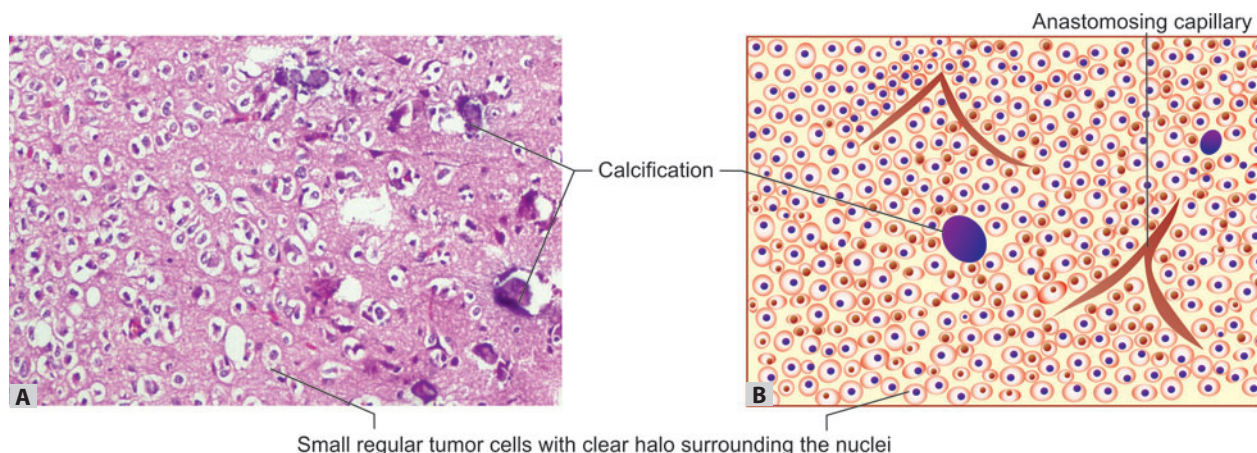
Ependymoma

Tumor arising from **ependymal cells**, which normally line ventricular system or central canal of the spinal cord. They usually arise next to the ependyma-lined ventricular system, including the oft-obliterated central canal of the spinal cord.

Sites

Most common site of ependymoma:

- In children: typically near fourth ventricle
- In adults: spinal cord.
- First 2 decades of life: **Fourth ventricle**.
- **Adults: Spinal cord**—frequently associated with neuro-fibromatosis type 2 (NF2).



Figs 28.6A and B: (A) (Photomicrograph); and (B) (Diagrammatic); Microscopy of oligodendroglioma. Tumor cells are small, round, regular, having clear cytoplasm forming "halos" around nuclei. The stroma shows thin-walled capillaries and foci of calcification

MORPHOLOGY**Gross**

Usually **solid and homogeneous** or papillary. **Moderately well demarcated** from adjacent brain parenchyma.

Microscopy (Fig. 28.7)**Ependymoma:**

- Ependymal rosettes
- Perivascular pseudorosettes.
- Tumor cells
 - **Resemble normal ependymal cells**
 - Cells are regular having well-defined cell membranes.
 - Nuclei are round to oval having abundant granular chromatin.
 - Variable amount of dense fibrillary background.
- **Rosettes** (Fig. 28.7)
 - **Ependymal rosettes:** Tumor cells may form **gland like round or elongated structures, which resemble the embryologic ependymal canal** → known as ependymal rosettes or canals, and show long, delicate processes extending into a lumen.
 - **Perivascular pseudorosettes:** Tumor cells are arranged **around vessels** to form perivascular pseudorosettes. More frequent than true ependymal rosettes.

Special stain

PTAH may reveal **PTAH-positive blepharoplasts**, which represent basal bodies of cilia of ependymal cells.

Immunohistochemistry

GFAP is positive in most ependymomas.

Grade: Most are **well differentiated** and behave as WHO grade II/IV lesions.

Clinical features: Posterior fossa ependymomas usually manifest with hydrocephalus secondary to obstruction.

Spread: Through CSF is common and is associated with a poor prognosis.

Embryonal Tumors

Embryonal tumors are of **neuroectodermal origin**, which consists of **primitive, undifferentiated cells**. The most common is the medulloblastoma.

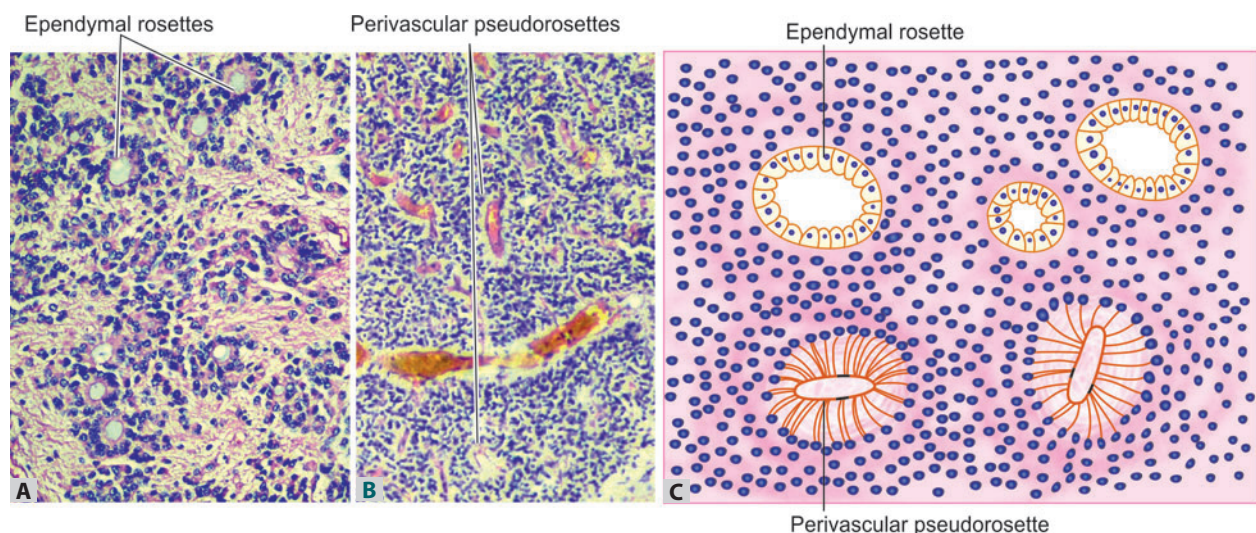
Medulloblastoma**Q. Write short note on medulloblastoma.**

- **Highly malignant undifferentiated or embryonal tumor.** Neuronal and glial markers may be expressed.
- **Age:** Occurs **predominantly in children** (majority at the end of the first decade). Constitutes about 20% of the brain tumors in children.

Molecular Genetics

Depending on the molecular alterations, medulloblastoma can be divided into four groups:

1. **WNT type:** It is characterized by mutations in the WNT signaling pathway. It develops in older children and shows classic histological features of medulloblastoma. It shows monosomy of chromosome 6 and nuclear expression of β -catenin. It has best prognosis.
2. **SHH type:** It is characterized by mutations involving the sonic hedgehog signaling pathway. It is seen in infants or young adults and histologically shows nodular



Figs 28.7A to C: Microscopic appearance of ependymoma (A and B): hematoxylin and eosin (H & E); and (C) diagrammatic, showing ependymal rosettes and perivascular pseudorosettes

desmoplastic appearance. It may show amplification of MYCN. Prognosis is intermediate between the WNT subtype and groups 3 and 4.

3. **Group 3 medulloblastoma:** It usually shows MYC amplification and isochromosome 17 (i17q). It usually found in infants and children. Microscopically, it may show a classic or large cell histology and has worst prognosis.
4. **Group 4:** It is characterized by an i17q cytogenetic alteration, classic or large cell histology and without MYC amplification. Prognosis is intermediate.

Medulloblastoma: Highly malignant undifferentiated/embryonal tumor predominant in children.

MORPHOLOGY

Tumors which histologically appear similar to medulloblastoma in sites other than CNS are known as primitive neuroectodermal tumor (PNET).

Medulloblastoma: Occurs exclusively in cerebellum.

Medulloblastoma: Most common site vermis of cerebellum (70%).

Medulloblastoma: Most common site in adults is lateral cerebellar hemisphere.

Site

Exclusively occurs in the **cerebellum**.

- **Children:** In the midline of the cerebellum
- **Adults:** Lateral locations in the cerebellar hemispheres

Gross

- Well circumscribed
- Gray and friable.

Microscopy (Fig. 28.8)

Medulloblastoma:

- Highly cellular
- Small cells with little cytoplasm
- Homer-Wright rosettes.

- **Highly cellular**, composed of **sheets of anaplastic (small blue) cells**.
- **Tumor cells: Small, with little cytoplasm and hyperchromatic nuclei** (elongated or crescent shaped).
- **Numerous mitotic figures**
- **Homer-Wright (neuroblastic) rosette:** It is characterized by **central neurophil** (delicate pink material formed by neuronal processes) **surrounded by primitive tumor cells** may be seen.

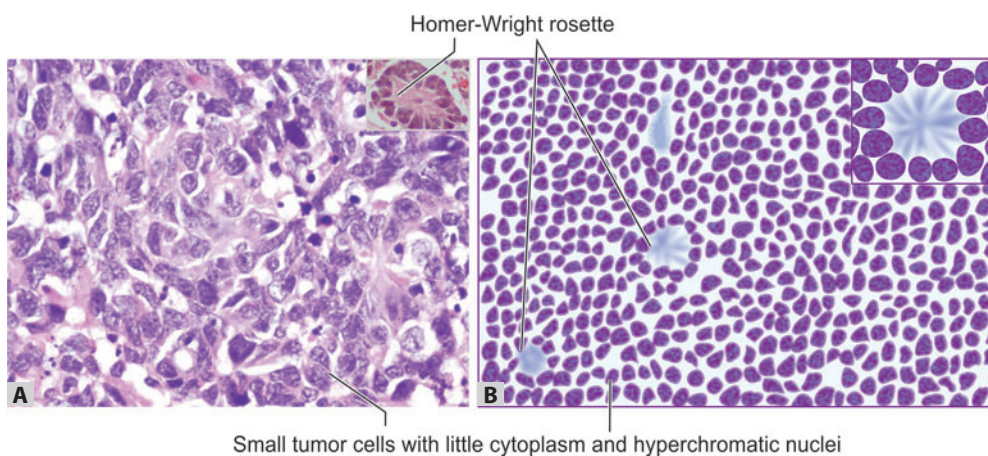
Immunohistochemistry: GFAP+.

Nodular/desmoplastic variant: Characterized by nodular, reticulin-free zones (pale islands) surrounded by densely packed highly proliferative tumor cells. These tumor cells have hyperchromatic and moderately pleomorphic nuclei and they produce a collagen and dense intercellular reticulin fiber network.

Large cell variant: It is characterized by monomorphic cells with large, round, vesicular nuclei, prominent nucleoli, and frequent mitoses and apoptotic cells. These cells show variable amount of eosinophilic cytoplasm.

Spread

- **Through the CSF is common**, may present as nodular masses anywhere in the CNS.
- **Metastases to the cauda equina** are sometimes termed **drop metastases**.



Figs 28.8A and B: Microscopic (A) Photomicrograph; (B) Diagrammatic appearance of medulloblastoma. Inset shows Homer-Wright rosette

Homer-Wright rosette:

- Medulloblastoma
- Neuroblastoma.

Medulloblastoma: Most radiosensitive brain tumor.

Medulloblastoma: Dissemination through CSF is common leading to drop metastasis.

Clinical Features

- Cerebellar dysfunction.
- **Hydrocephalus** due to occlusion of CSF flow caused by rapid growth of tumor.

Prognosis

- **Poor** for untreated patients.
- **MYC amplification** is associated with **poor prognosis**.

MENINGIOMAS

Q. Write short note on meningioma.

Meningiomas are **benign intracranial tumors** that arise from the **meningothelial cell** of the arachnoid matter.

Incidence: 20% of all primary intracranial neoplasms.

Age: Peak during **fourth to fifth decades**.

Sex: **Female predominance** (female-to-male ratio is 3:2).

Meningioma:

- Benign tumor of meningotheial cell
- Female predominance.

Molecular Genetics

Most consistent cytogenetic abnormality is deletion/loss of chromosome 22 (especially the long arm-22q). Deletions of the region of 22q12 which contains the *NF2* gene (encodes the protein merlin) are a common meningioma developing in mutation of *NF2* gene. In sporadic meningiomas 50–60% has mutations in the *NF2* gene. Higher grade meningiomas are more often associated with *NF2* mutations, loss of chromosome 22, and chromosomal instability.

Meningioma: Most common intracranial, extra-axial dural-based neoplasms.

MORPHOLOGY

Q. Write short note on morphology of meningioma.

Site

Anywhere in intracranial site both on external surfaces of the brain as well as within the ventricular system. **Most common sites** include **parasagittal regions of the cerebral hemispheres**, dura over the **lateral convexity**, **olfactory groove**, etc.

Gross

- **External surface:** **Well-circumscribed** (usually encapsulated), **smooth, rounded, bosselated** or **polypoid masses** (Fig. 28.9)
- **Size:** **Variable**
- **Consistency:** Range from **firm** and fibrous to finely gritty (in the presence of psammoma bodies).
- **Cut surface:** **Gray without necrosis or hemorrhage.**
- **Base:** **Usually attached to the dura.** They compress the underlying brain but do not infiltrate.

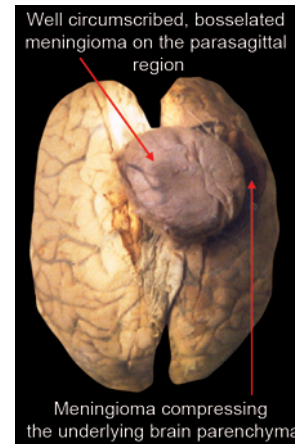


Fig. 28.9: Meningioma on the parasagittal region of the cerebral hemispheres

Microscopy (Fig. 28.10)

The **characteristic features** of meningiomas are a **whorled pattern** of arrangement of meningotheial cells and the presence of **psammoma bodies** (laminated, spherical calcospherites).

Meningioma:

- Whorled pattern of arrangement of tumor cells
- May show psammoma bodies.

Types

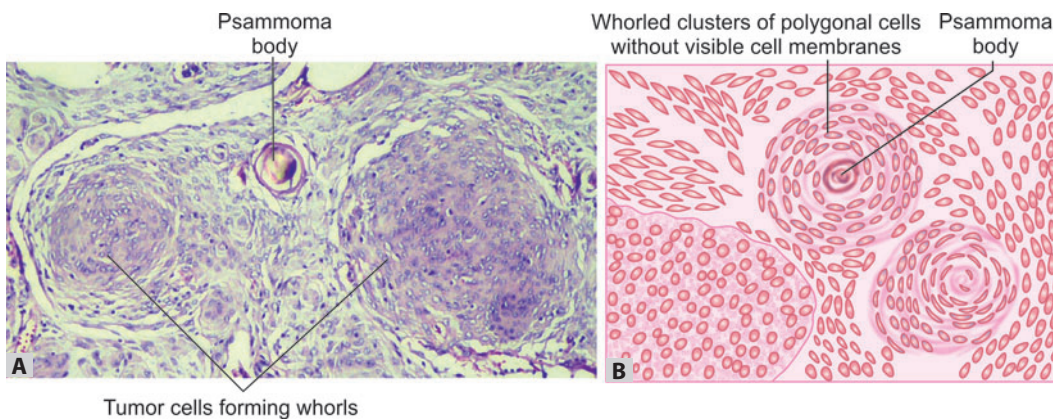
Q. Write short note on histological subtypes of meningioma.

Various histological types do not differ in the biological behavior and has no prognostic significance.

- **Syncytial (meningotheial):** It shows **whorled clusters of polygonal cells without visible cell membranes** (syncytial). The tumor cells have **centrally placed oval nuclei**.
- **Fibroblastic:** It consists of **spindle-shaped elongated cells**, which are **arranged in the interlacing or parallel bundles** with abundant collagen deposition in between the cells.
- **Transitional/mixed:** It shows **features of the syncytial and fibroblastic types**. The tumor cells show whorled pattern often around a central capillary-sized blood vessel. The center of some of the whorls **may show psammoma bodies**.
- **Psammomatous:** It shows **numerous psammoma bodies**.
- **Secretory:** It consists of **gland-like PAS-positive, eosinophilic secretions** (pseudopsammoma bodies).
- **Microcystic:** It is composed of **microcystic spaces** with a loose, spongy appearance.
- **Angiomatous:** It shows numerous blood vessels.

Immunohistochemistry: **Negative for GFAP and keratins**, but **positive for epithelial membrane antigen**

Grade: Most meningiomas are considered as WHO grade I/IV.



Figs 28.10A and B: Microscopy of meningioma. (A) Hematoxylin and eosin (H & E); (B) Diagrammatic, showing tumor cells with indistinct cytoplasm arranged in whorls

Meningioma: Contain calcified bodies (dystrophic calcification) called psammoma bodies.

Meningioma: Psammoma body is a concentric calcium deposit (dystrophic calcification).

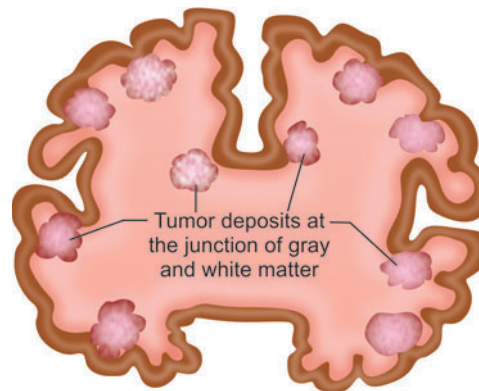
Atypical Meningioma (WHO Grade II/IV)

It is **locally aggressive tumor** with a **higher rate of recurrence** than meningioma.

- They show either four or more mitoses/10 high power fields or at least 3 atypical features (increased cellularity, small cells with a high nuclear-to-cytoplasmic ratio, prominent nucleoli, or necrosis).

Anaplastic (Malignant) Meningioma (WHO Grade III/IV)

It is a highly aggressive tumor and histologically appear like high-grade sarcoma.



Metastatic tumors are most common intracranial tumors.

Lung cancer: Most common cancer causing metastasis to the brain.

Fig. 28.11: Sites of deposit of metastatic tumors in brain

Clinical Features

Meningiomas are **usually slow-growing tumors**. They produce **symptoms by compressing underlying brain tissue** and depend on the site.

METASTATIC TUMORS

Choriocarcinoma has high likelihood of metastasizing to brain whereas carcinoma prostate almost never grow in the brain.

Metastatic tumors are the **most common intracranial neoplasms**.

Primary site: Mostly carcinomas and the **five most common primary sites are:** 1) lung, 2) breast, 3) skin (melanoma), 4) kidney, and 5) gastrointestinal tract.

Route of spread: Through the bloodstream, generally in patients with advanced cancer.

MORPHOLOGY

Metastatic tumors: Most common primary sites are:

1. Lung
2. Breast
3. Skin (melanoma)
4. Kidney
5. Gastrointestinal tract.

Gross

- Intraparenchymal metastases in contrast with a primary glioma, form **multiple, sharply demarcated masses** (Fig. 28.11), usually surrounded by a prominent zone of edema.
- The **boundary between metastatic tumor and surrounding brain parenchyma is sharp** and well defined both grossly and microscopically.
- They are **usually seen at the junction of gray matter and white matter**.

Microscopy

It is similar to that of primary tumor.

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Appendices

APPENDIX 1: VARIOUS IMPORTANT BODIES AND ITS ASSOCIATED CONDITIONS

Various Important Bodies and its Associated Conditions

<i>Name of the body</i>	<i>Associated condition</i>
Aschoff body	Rheumatic fever
Asbestos body	Asbestosis
Ferruginous body	
Asteroid body	Sarcoidosis and sporotrichosis
Call-Exner body	Granulosa cell tumor
Civatte (colloid) body	Lichen planus
Councilman body	Acute hepatitis
Creola body	Asthma
Donovan body	Granuloma inguinale
Gamna Gandy body	Congestive splenomegaly
Halberstaedter-Prowazek's (HP) body	Trachoma
Heinz body	G6PD deficiency
Hirano body	Alzheimer disease
Howell-Jolly body	After splenectomy, asplenia, megaloblastic anemia, severe hemolytic anemia
Leishman-Donovani (LD) body	Kala-azar
Lewy body	Parkinsonism
Mamillary body (Rokitansky tubercle)	Benign cystic teratoma of ovary
Michaelis-Gutmann body	Malakoplakia
Mallory bodies	Alcoholic hepatitis, Wilson disease, hepatocellular carcinoma, primary biliary cirrhosis, etc.
Negri body	Rabies (intracytoplasmic)
Pick body	Pick disease
Psammoma body (calcospherites)	Papillary carcinoma of thyroid, serous papillary cystadenoma and papillary carcinoma of ovary, meningioma, papillary renal cell carcinoma (RCC)
Russell body (cytoplasmic) and Dutcher body (nuclear)	Multiple myeloma
Schaumann body	Sarcoidosis
Schiller-Duval body	Yolk sac (endodermal sinus) tumor
Verocay body ("Antoni A")	Neurilemmoma (Schwannoma)
Weibel-Palade body	Endothelial cells
Zebra body	Metachromatic leukodystrophy

APPENDIX 2: IMPORTANT CELLS IN VARIOUS LESIONS AND PATHOGNOMONIC STRUCTURES IN DISEASES

Important Cells in Various Lesions

<i>Name of the cell</i>	<i>Associated conditions</i>
Anitschkow cell (plump activated macrophage)	Rheumatic heart disease
Burr cell/helmet cell/triangle cell	Uremia, HUS, MAHA (microangiopathic hemolytic anemia)
Bite cell	G6PD deficiency
Foam cell (lipid-filled macrophage)	Atheromatous plaque, storage disorders, xanthoma
Flame/mott cell (plasma cells with glycoprotein globules)	Multiple myeloma
Glitter cell (leukocyte with visible movement of cytoplasmic process)	Pyelonephritis
Reed sternberg cell, Hodgkin cell, lacunar cell, mummified cell	Hodgkin lymphoma
Hürthle cells	Hashimoto thyroiditis, Hürthle cell adenoma of thyroid
Ito cell (space of Disse in liver) stores vitamin A	Secrets collagen in cirrhosis
LE cell (neutrophil with phagocytosed nuclear chromatin)	Systemic lupus erythematosus (SLE)
Tart cell	
Langerhans cell	Antigen presenting cell in the epidermis
Merkel cell (present in lower layer of epidermis)	Merkel cell carcinoma
Target cell	Thalassemia, HbS, HbC, liver disease

Pathognomonic Structures in Diseases

<i>Pathognomonic characteristic</i>	<i>Disease</i>
Aschoff body and Anitschkow cell	Rheumatic fever
Auer rod in myeloblast	Acute myeloid leukemia
Cytoplasmic Birbeck granules on ultrastructural examination	Langerhans cell histiocytosis
Mosaic pattern of lamellar bone	Paget disease of bone
Negri body	Rabies
Owl's eye appearance of intranuclear inclusion body	Cytomegalovirus
Proliferation center	Chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL)
Reed-Sternberg (RS) cell	Hodgkin lymphoma
Spongiform transformation of the cerebral cortex	Creutzfeldt-Jakob disease
Tophi	Gout
Warthin-Finkeldey cell	Measles
White blood cell (WBC) cast	Pyelonephritis

APPENDIX 3: LABORATORY VALUES OF CLINICAL IMPORTANCE

In this appendix, tables of reference values of some important common laboratory investigations are provided which will help in interpreting the results during examinations as well as during clinician practice. The term 'reference values' has replaced older terminology 'normal values/ranges'. A variety of factors can influence reference values and it varies between laboratories depending on the laboratory methods, mode of standardization and other factors. This is especially the case with enzyme assays. The reference or "normal" ranges given in this appendix may, therefore, not be appropriate for all laboratories and they should only be used as general guidelines. Hence, reference values provided by the laboratory performing the test should be used in the interpretation of laboratory results. Most clinical laboratories and all medical and scientific journals use SI system. Since, conventional units are still used in many laboratories in many developing countries, in this section, laboratory values are given in both conventional and international units. Many analytes are measured in either serum (the supernatant of clotted blood) or plasma (the supernatant of anticoagulated blood).

The laboratory reference values in this appendix is divided into different section namely: (1) hematology and coagulation (Table A-3.1), (2) clinical chemistry of blood (Table A-3.2), (3) lipid profile (Table A-3.3), (4) urea and electrolytes (Table A-3.4), (5) thyroid function tests (Table A-3.5), (6) urine (Table A-3.6), and (7) cerebrospinal fluid (Table A-3.7).

Hematology and Coagulation (Table A-3.1)

TABLE A-3.1: Hematology and coagulation

Component (specimen)	Reference value	
	Conventional	SI units
RBCs and hemoglobin		
RBC count		
• Males	4.5–5.5 × 10 ¹² /L (mean 5.0 × 10 ¹² /L)	
• Females	3.8–4.8 × 10 ¹² /L (mean 4.3 × 10 ¹² /L)	
RBC diameter	6.7–7.7 µm (mean 7.2 µm)	
RBC indices (absolute values)		
• Mean corpuscular volume (MCV)	82–100 fL	
• Mean corpuscular hemoglobin (MCH)	27–32 pg	
• Mean corpuscular hemoglobin concentration (MCHC)	31–35 g/dL	
• Red cell distribution width (RDW)	11.5–14.0%	
RBC lifespan	120 days	
Erythrocyte sedimentation rate (ESR) (Whole blood)		
• Westergren, 1st hour		
– Males	0–15 mm 1st hour	
– Females	0–20 mm 1st hour	
– Children	0–10 mm 1st hour	
• Wintrobe, 1st hour		
– Males	0–9 mm 1st hour	
– Females	0–20 mm 1st hour	
Ferritin (serum)		
• Males	20–300 ng/mL	20–300 µg/L
• Females	15–200 ng/mL	15–200 µg/L
Folate (serum)	3–20 µg/L	3–20 ng/mL
Hematocrit (PCV)		
• Males	38–47%	

Contd...

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Component (specimen)	Reference value	
	Conventional	SI units
• Females	36–46%	
• Infants (cord blood)	45–70%	
Haptoglobin (serum)	40–240 mg/dL	0.4–2.4 g/L
Hemoglobin (Hb)		
• Adult hemoglobin (HbA)	95–98%	
• Males	13.0–17.0 g/dL	
• Females	12.0–15.0 g/dL	
• Hemoglobin A ₂ (HbA ₂)	1.5–3.5%	
• Hemoglobin, fetal (HbF) in adults	<0–2%	
• HbF, children under 6 months	<5%	
Iron, total (serum)	50–150 µg/dL	7–25 µmol/L
• Total iron binding capacity (TIBC)	310–340 µg/dL	45–73 µmol/L
• Iron saturation	20–45%	0.20–0.45
Osmotic fragility		
• Slight hemolysis	at 0.45 to 0.39 g/dL NaCl	
• Complete hemolysis	at 0.33 to 0.36 g/dL NaCl	
• Mean corpuscular fragility	0.4–0.45 g/dL NaCl	
Reticulocytes		
• Adults	0.5–2.5%	
• Infants	2–6%	
• Newborn (cord blood)	1–7%	
Transferrin saturation		
• Male	25–56%	
• Female	14–51%	
Vitamin B ₁₂ (serum)		
• Body stores	10–12 mg	
• Daily requirement	2–3 µg	
• Serum level	280–1,000 pg/mL	
Auto hemolysis test (whole blood)	0.4–4.50%	0.004–0.045
Autohemolysis test with glucose (whole blood)	0.3–0.7%	0.003–0.007
Leukocytes		
Differential leukocyte count (DLC)		
• P (polymorphs or neutrophils)	40–70% (2,000–7,500/µL)	
• L (lymphocytes)	20–40% (1,500–4,000/µL)	
• M (monocytes)	2–10% (200–800/µL)	
• E (eosinophils)	1–6% (40–450/µL)	
• B (basophils)	<1% (10–100/µL)	
Total leukocyte count (TLC)		
• Adults	4,000–11,000/µL	

Contd...

Contd...

Component (specimen)	Reference value	
	Conventional	SI units
• Infants (full term, at birth)	10,000–25,000/ μ L	
• Infants (1 year)	6,000–16,000/ μ L	
Platelets and coagulation		
Bleeding time (BT)		
• Ivy's method	2–7 minutes	
• Template method	2–9 minutes	
Clot retraction time (clotted blood)		
• Qualitative	Visible in 60 minutes (complete in <24 hours)	
• Quantitative	48–64% (55%)	
Clotting time (CT)		
• Lee and White method	4–11 minutes	
D-dimer (plasma)	220–740 ng/mL	
Fibrinogen (plasma)	200–400 mg/dL	
Fibrin split (or degradation) products (FSP or FDP)	<10 μ g/mL	<10 mg/L
Partial thromboplastin time with kaolin (PTTK) or activated partial thromboplastin time (APTT/aPTT)	30–40 second	
Platelet count	150,000–450,000/ μ L	
Prothrombin time (PT) (Quick's one stage method)	11–16 second	
Thrombin time (TT)	15–19 second (control \pm 2 sec)	

Clinical Chemistry of Blood (Table A-3.2)

TABLE A-3.2: Clinical chemistry of blood

Component	Specimen	Reference value	
		Conventional	SI units
Alpha fetoprotein (AFP), adults	Serum	0–8.5 ng/mL	0–8.5 μ g/L
Aminotransferases (transaminases)			
• Aspartate (AST, SGOT)	Serum	12–38 U/L	0.20–0.65 μ kat/L
• Alanine (ALT, SGPT)	Serum	7–41 U/L	0.12–0.70 μ kat/L
Amylase	Serum	20–96 U/L	0.34–1.6 μ kat/L
Bilirubin	Serum		
• Total		0.3–1.3 mg/dL	5.1–22 μ mol/L
• Direct (conjugated)		0.1–0.4 mg/dL	1.7–6.8 μ mol/L
• Indirect (unconjugated)		0.2–0.9 mg/dL	3.4–15.2 μ mol/L
CA 125	Serum	0–35 U/mL	0–35 Ku/L
Calcium—ionized	Whole blood	4.5–5.3 mg/dL	1.12–1.32 mmol/L
Calcium—total	Serum	8.7–10.2 mg/dL	2.2–2.6 mmol/L
Chloride	Serum	102–109 mEq/L	102–109 mmol/L
C-reactive proteins	Serum	0.2–3.0 mg/L	0.2–3.0 mg/L

Contd...

Contd...

Component	Specimen	Reference value	
		Conventional	SI units
Creatine kinase (CK), total	Serum		
• Males		51–294 U/L	0.87–5.0 μ kat/L
• Females		39–238 IU/L	0.66–4.0 μ kat/L
Creatine kinase MB (CKMB)	Serum	0–5.5 ng/mL	0–5.5 μ g/L
Gamma glutamyl transpeptidase (transferase) (γ -GT)	Serum	9–58 IU/L	0.15–1.00 μ mol/L
Glucose (fasting)	Plasma		
• Normal		70–100 mg/dL	<5.6 mmol/L
• Impaired fasting glucose (IFG)		101–125 mg/dL	5.6–6.9 mmol/L
• Diabetes mellitus		>126 mg/dL	>7.0 mmol/L
Glucose (2-hour postprandial)	Plasma		
• Normal		<140 mg/dL	<7.8 mmol/L
• Impaired glucose tolerance (IGT)		140–200 mg/dL	7.8–11.1 mmol/L
• Diabetes mellitus		>200 mg/dL	>11.1 mmol/L
Glycated hemoglobin (HbA _{1c})	Whole blood	4.0–6.0%	20–42 mmol/mol Hb
Lactate dehydrogenase (LDH)	Serum	115–221 U/L	2.0–3.8 μ kat/L
Muramidase	Serum	5–20 μ g/mL	
5-nucleotidase	Serum	0–11 U/L	0.02–0.19 μ kat/L
Phosphatases	Serum		
• Acid phosphatase		0–5.5 U/L	0.90 μ kat/L
• Alkaline phosphatase		33–96 U/L	0.56–1.63 μ kat/L
Prostate-specific antigen (PSA)	Serum	0–4.0 ng/mL	0–4.0 μ g/L
Proteins—total	Serum	6.7–8.6 g/dL	67–86 g/L
• Albumin		3.5–5.5 g/dL	35–55 g/L
• Globulins		2.0–3.5 g/dL	20–35 g/L
• Albumin/globulin ratio		1.5–3 : 1	
Rheumatoid factor	Serum	<15 IU/mL	< 15 kIU/L
Troponins, cardiac (cTn)			
• Troponin I (cTnI)	Serum	0–0.08 ng/mL	0–0.8 μ g/L
• Troponin T (cTnT)	Serum	0–0.01 ng/mL	0–0.1 μ g/L
Urea nitrogen (BUN)	Blood	7–20 mg/dL	2.5–7.1 mmol/L
Uric acid	Serum		
• Males		3.1–7.0 mg/dL	0.18–0.41 μ mol/L
• Females		2.5–5.6 mg/dL	0.15–0.33 μ mol/L

Lipid Profile (Table A-3.3)**TABLE A-3.3:** Lipid profile

Component	Reference value	
	Conventional	SI units
Total serum cholesterol		
• Desirable for adults	<200 mg/dL	<5.17 mmol/L
• Borderline high	200–239 mg/dL	5.17–6.18 mmol/L
• High undesirable	>240 mg/dL	>6.21 mmol/L
LDL cholesterol		
• Desirable range	100–130 mg/dL	<3.34 mmol/L
• Borderline high	130–159 mg/dL	3.36–4.11 mmol/L
• High	160–189 mg/dL	4.11–4.20 mmol/L
• Very high	>190 mg/dL	>4.21 mmol/L
HDL cholesterol		
• Low	<40 mg/dL	<1.03 mmol/L
• High, protective range	>60 mg/dL	>1.55 mmol/L
Triglycerides	<160 mg/dL	<2.26 mmol/L

Urea and Electrolytes (Table A-3.4)**TABLE A-3.4:** Urea and electrolytes

Analyte	Reference value	
	Conventional	SI units
Sodium	136–146 mEq/L	136–146 mmol/L
Potassium	3.5–5.0 mEq/L	3.5–5.0 mmol/L
Chloride	95–107 mEq/L	95–107 mmol/L
Urea	20–40 mg/dL	3.3–6.6 mmol/L
Creatinine	0.6–1.2 mg/dL	53–106 μ mol/L

Thyroid Function Tests (Table A-3.5)**TABLE A-3.5:** Thyroid function tests

Thyroid function tests	Specimen	Reference value	
		Conventional	SI units
Radioactive iodine uptake (RAIU) 24 hours		5–30%	
Thyroxine (T ₄) total	Serum	5.4–11.7 μ g/dL	70–151 nmol/L
Triiodothyronine (T ₃) total	Serum	77–135 ng/dL	1.2–2.1 nmol/L
Thyroid-stimulating hormone (TSH)	Serum	0.4–4.25 μ U/mL	0.4–4.25 mU/L

Urine (Table A-3.6)**TABLE A-3.6:** Normal urine values

<i>Component</i>	<i>Reference value</i>
Volume—24 hours	600–1800 mL (variable)
pH	5.0–9.0
Specific gravity, quantitative (random)	1.002–1.028 (average 1.018)
Protein—24 hours urine	<150 mg/day
Protein, qualitative (random)	Negative
Glucose, quantitative—24 hours urine	50–300 mg/day
Glucose, qualitative (random)	Negative
Urobilinogen—24 hour urine	1.0–3.5 mg/day
Microalbuminuria (24 hours)	0–30 mg/24 hours (0–0.03 g/day) (0–30 µg/mg creatinine) (0–0.03 g/g creatinine)

Cerebrospinal Fluid (Table A-3.7)**TABLE A-3.7:** Normal values of cerebrospinal fluid

<i>Component</i>	<i>Reference value</i>	
	<i>Conventional</i>	<i>SI units</i>
Cerebrospinal fluid volume	120–150 mL	
Appearance	Clear and colorless	
Cerebrospinal fluid pressure	60–150 mm water	
pH	7.31–7.34	
Total proteins	20–40 mg/dL	0.14–0.45 g/L
Glucose	40–80 mg/dL	2.3–4.5 mmol/L
Chlorides	720–750 mg/dL	
Cells		
• Polymorphs	Usually absent	
• Lymphocytes	0–5/µL	

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